U.S. Department of Commerce Patent and Trademark Office		Attorney's Docket No. 4894
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. Application No. (if known, see 37 CFR 1.5) 09/623326
INTERNATIONAL APPLICATION NO. PCT/EP99/01674	INTERNATIONAL FILING DATE 15 March 1999	PRIORITY DATE CLAIMED 13 March 1998

TITLE OF INVENTION
POLYMERASE CHIMERAS

APPLICANT(S) FOR DO/EO/US
FREY, VILLBRANDT, SCHOMBURG, SOBEK AND ANKENBAUER

Applicants herewith submit to the United States Designated/Elected Office (DO/EO/US) the following items and other information

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C.371
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 3I5 U.S.C. 371
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] has been transmitted by the International Bureau
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US)
6. [X] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [X] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [X] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). [unexecuted]
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 16 below concern other document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.

14. [ ] A substitute specification.

15. [ ] A change of power of attorney and/or address letter.

16. Other items or information:

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) 09/623326	INTERNATIONAL APPLICATION NO. PCT/EP99/01674	ATTORNEY'S DOCKET NUMBER 4894

17. [X] The following fees are submitted:

Basic National Fee (37 CFR 1.492(a)(1)-(5)):
Search Report has been prepared by the EPO or JPO..... .$840.00

International preliminary examination fee paid to USPTO (37 CFR 1.482) $670.00

No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(..... .$760.00

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR1.445(a)(2)) paid to USPTO..... .$970.00

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)..... .$96.00

CALCULATIONS/PTO USE ONLY

ENTER APPROPRIATE BASIC FEE AMOUNT =				$ 840.00	
Surcharge of $130.00 for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$	
Claims	Number Filed	Number Extra	Rate		
Total claims	36 - 20 =	16	X18.00	$ 288.00	
Independent claims	6 - 3 =	3	X78.00	$ 234.00	
Multiple dependent claim(s) (if applicable)			+$260.00	$260.00	
TOTAL OF ABOVE CALCULATIONS =				$ 840.00	
Reduction by ½ for filing small entity, if applicable. Verified Small Entity Statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =					
Processing fee of $130.00 for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$1,622.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$	
TOTAL FEES ENCLOSED =				$	
			Amount to be refunded :		$
			charged		$

a. [ ] A check in the amount of $_____ to cover the above fees is enclosed.

b. [X] Please charge my Deposit Account No. 500812 in the amount of $ 1,622.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 500812. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Victor K. Lee, Ph.D.
Roche Molecular Systems, Inc.
1145 Atlantic Avenue
Alameda, CA 94501

Express Mail No. EK772695187US
Date: August 30, 2000

SIGNATURE

VICTOR K. LEE, Ph.D.
NAME

35,750
REGISTRATION NUMBER

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application
based on International Application No. PCT/EP99/01674
Filed March 15, 1999
Inventor(s) FREY *et al.*

For: **POLYMERASE CHIMERAS**

Attorney Docket No. 4894

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
BOX PCT
Washington, D.C. 20231

Alameda, CA 94501
Date: August 30, 2000

Sir:

Prior to examining the above-referenced application as entering the National Stage under 35 U.S.C. §371, please consider the following amendments and remarks.

IN THE CLAIMS

At page 1, line 1 of the Amendment of claims under PCT Article 19, please delete "Claims" and insert therefor --WHAT IS CLAIMED IS--.

Please amend the claims as follows:

1. (Amended) A polymerase [Polymerase] chimera comprising [composed of] functional amino acid fragments of at least two different polymerases, wherein the functional amino acid fragments are active in the polymerase chimera, a [and the] domain having polymerase activity is derived from the first polymerase and a [the] domain having 3'- 5' exonuclease activity is derived from the second polymerase, and wherein the amino acid sequence of the polymerase chimera essentially corresponds to SEQ ID NO: 8.

2. (Amended) A polymerase [Polymerase] chimera comprising [composed of] functional amino acid fragments of at least two different polymerases, wherein the functional amino acid fragments are active in the polymerase chimera, a [and the] domain having polymerase activity is derived from the first polymerase and a [the] domain having 3'- 5' exonuclease activity is derived from the second polymerase, and wherein the amino acid sequence of the polymerase chimera essentially corresponds to SEQ ID NO: 10.

3. (Amended) A polymerase [Polymerase] chimera comprising [composed of] functional amino acid fragments of at least two different polymerases, wherein the functional amino acid fragments are active in the polymerase chimera, a [and the] domain having polymerase activity is derived from the first polymerase and a [the] domain having 3'- 5' exonuclease activity is derived from the second polymerase, and wherein the amino acid sequence of the polymerase chimera essentially corresponds to SEQ ID NO: 12.

4. (Amended) The polymerase [Polymerase] chimera as claimed in one of the claims 1-3, wherein the chimera additionally has reverse transcriptase [RT] activity.

5. (Amended) The polymerase [Polymerase] chimera of claim 4 [as claimed in one of the claims 1-4], wherein histine tags have been incorporated into the amino acid sequence of the chimera.

6. (Amended) A nucleic acid that encodes the [DNA sequence of a] polymerase chimera as claimed in claim 1, 2 or 3 [one of the claims 1-5].

7. (Amended) A nucleic acid that encodes [DNA sequence of] a polymerase chimera comprising the sequence of SEQ ID NO. 2 [according to SEQ ID N0. 2].

8. (Amended) A nucleic acid that encodes [DNA sequence of] a polymerase chimera comprising the sequence of SEQ ID NO. 4 [according to SEQ ID NO. 4].

9. (Amended) A nucleic acid that encodes [DNA sequence of] a polymerase chimera comprising the sequence of SEQ ID NO. 6 [according to SEQ ID NO. 6].

10. (Amended) A vector comprising the nucleic acid [Vector containing a DNA sequence] as claimed in claim 6 [claims 6-9].

11. (Amended) A host [Transformed] cell which has been transformed with [contains] the vector as claimed in claim 10.

12. (Amended) A process [Process] for the production of the polymerase chimeras as claimed in one of the claims 1-3 [1-5], wherein the process comprises the following steps:

- (a) designing variants with the aid of amino acid sequence alignments, of three dimensional [3D] models or with the aid of experimentally determined three dimensional [3D] structures;

- (b) production of domain exchange variants by genetic engineering;

- (c) ligating [the] DNA fragments that encode the variants into starting vectors;

- (d) expression of the chimeras in a host which has been [was] transformed by vectors carrying the DNA fragments; and

- (e) purifying the expressed polymerase chimeras.

13. (Amended) A method for using [Use of] the polymerase chimeras as claimed in one of the claims 1-3 [1-5] for PCR.

14. (Amended) A method for using [Use of] the polymerase chimeras as claimed in one of the claims 1-3 for sequencing [1-5 to sequence] DNA fragments.

15. (Amended) A method for using [Use of] the polymerase chimeras as claimed in one of the claims 1-3 [1-5] for RT-PCR starting with an RNA template.

16. (Amended) A kit comprising [Kit containing] a polymerase chimera as claimed in one of the claims 1-3 [1-5].

REMARKS

Applicants have amended the claims to comply with the U.S. patent practice in matters of form and to remove multiple dependency in certain claims. After entry of this Amendment, claims 1-16 are pending in this application. The amendments are fully supported by the specification, and do not introduce new matter. Entry of this Amendment is respectfully requested.

The total filing fee on the Transmittal Letter To The United States Designated/Elected Office (DO/EO/US) Concerning A Filing Under 35 U.S.C. §371 is calculated on the basis of this Amendment.

Respectfully submitted:

By: _____

Victor K. Lee, Ph.D.
Agent for Applicants
Reg. No. 35,750
1145 Atlantic Avenue
Alameda, CA 94501
Telephone (510) 814-2966
Telefax (510) 814-2973

Polymerase chimeras

The invention concerns polymerase chimeras which are composed of amino acid fragments representing domains and which combine properties of naturally occurring polymerases that are advantageous with regard to a particular application. It has surprisingly turned out that the domains from the various enzymes are active in the chimeras and exhibit a cooperative behaviour. The present invention especially concerns those polymerase chimeras in which the domains having polymerase activity and domains having 3'-5'exonuclease activity are derived from different enzymes. Such chimeras can also have RT activity. In addition the present invention concerns a process for the production of the chimeras according to the invention and the use of these chimeras for the synthesis of nucleic acids e.g. during a polymerase chain reaction. Moreover the present invention concerns a kit which contains the polymerase chimeras according to the invention.

According to Braithwaite, D.K. and Ito, J. (1993) Nucl. Acids Res. 21, 787-802 DNA polymerases are divided according to the correspondence in their amino acid sequences into three main families with subclasses. Joyce, C.M. and Steitz, T.A. (1994) Annu. Rev. Biochem. 63, 777-822 give a summary of the motifs and conserved amino acids that were found. In prokaryotes the main distinction is made between three polymerases: polymerase I, II and III. These polymerases differ with regard to their function in the cell and with regard to

their properties. DNA polymerase I is considered to be a repair enzyme and frequently has 5'-3' as well as 3'-5' exonuclease activity. Polymerase II appears to facilitate DNA synthesis which starts from a damaged template strand and thus preserves mutations. Polymerase III is the replication enzyme of the cell, it synthesizes nucleotides at a high rate (ca. 30,000 per minute) and is considered to be very processive. Polymerase III has no 5'-3' exonuclease activity. Other properties of polymerases are due to their origin such as e.g. thermostability or processivity.

Particular properties of polymerases are desirable depending on the application. For example thermostable, high-fidelity (i.e. polymerases with proof-reading activity), processive and rapidly synthesizing polymerases are preferred for PCR. Enzymes are preferred for sequencing which do not discriminate much between dideoxy and deoxy nucleotides. In contrast the proof-reading activity of polymerases, i.e. 3'-5' exonuclease activity, is not desirable for sequencing. For some applications e.g. PCR it is desirable that the polymerase has no or little 5'-3' exonuclease activity (5' nuclease activity).

Polymerases can also differ in their ability to accept RNA as a template i.e. with regard to their reverse transcriptase (RT) activity. The RT activity may be dependent on the presence of manganese or/and magnesium ions. It is often desirable that the RT activity of the polymerase is independent of manganese ions since the reading accuracy of polymerase is decreased in the presence of manganese ions. Polymerases additionally differ in their processivity which is also a desirable property for many applications.

There is therefore a need to optimize the properties of polymerases with regard to a particular application. In the past this was often achieved by introducing mutations or by deleting functions of the polymerases.

Thus for example the 5'-3' exonuclease activity was abolished by introducing mutations (Merkens, L.S. (1995) *Biochem. Biophys. Acta* 1264, 243-248) as well as by truncation (Jacobsen, H. (1974) *Eur. J. Biochem.* 45, 623-627; Barnes, W.M. (1992) *Gene* 112, 29-35). The ability of polymerases to discriminate between dideoxy and deoxynucleotides was reduced by introducing point mutations (Tabor S. and Richardson, C.C. (1995) *Proc. Natl. Acad. Sci.* 92, 6339-6343). Tabor and Richardson describe the construction of active site hybrids.

The object to provide polymerases with optimized properties was achieved by the present invention for the first time by producing polymerase chimeras by exchanging domains that are structurally and functionally independent of one another. Domains in the sense of the present invention are understood as regions which contain all essential centres or all functionally important amino acids such that the domains essentially retain their function. It is therefore also possible to exchange only parts i.e. functioning fragments of domains. Thus these domains can be referred to as functional amino acid fragments in the sense of the present invention. Furthermore the chimeras can be additionally modified by mutations or truncations. If it appears to be advantageous it is also possible to introduce mutations into the chimeras which further optimize their properties with regard to the respective application. Thus for example mutations can be introduced which reduce the ability of the polymerases

to discriminate between dideoxy and deoxy nucleotides. Alternatively desired properties such as processivity can be strengthened or introduced by introducing mutations or by truncation. The introduction of mutations or truncations can also abolish undesired properties e.g. the 5' nuclease activity.

Thus polymerase chimeras are a subject matter of the present invention which combine advantageous properties of naturally occurring polymerases with regard to a particular application. The polymerase chimeras according to the invention are composed of functional amino acid fragments of different enzymes which preferably represent domains of different enzymes. The invention surprisingly showed that the domains from the different enzymes are active in the chimera and exhibit a cooperative behaviour between the domains. The present invention also concerns general processes for the production of polymerase chimeras with optimized properties. This process according to the invention thus enables a chimera to be designed from an arbitrary combination of enzymes by exchanging domains. It is additionally preferred that the interactions at the sites of contact between the domains are further harmonized by various methods. This can for example lead to an increase in the thermostability of the chimeras. A further subject matter of the invention is a kit for the synthesis of nucleic acids which contains a chimera according to the invention.

Thermostable DNA polymerases with proof-reading function are being increasingly used in practice for PCR. The use of mixtures of *Taq* polymerase and thermostable proof-reading DNA polymerase (such as *Pfu*, *Pwo*, *Vent* polymerase) has proven to be particularly successful for

the amplification of long DNA molecules. Thus a further subject matter of the present invention was to combine the high processivity and thermostability of *Taq* polymerase with the 3'-5' exonuclease activity of another DNA polymerase in one enzyme. Hence the present invention especially concerns thermostable polymerase chimeras which have a processivity which corresponds to at least that of *Taq* polymerase and have a low error rate when incorporating nucleotides into the polymer chain during amplification due to the presence of a 3'-5' exonuclease activity (proof-reading activity). The combination of these two properties enables for example a chimera to be generated which is able to make long PCR products i.e. nucleic acid fragments which are larger than 2 kb. The chimera according to the invention is also suitable for amplifying shorter fragments.

The present invention therefore concerns in particular a polymerase chimera which is composed of functional amino acid fragments of two different polymerases wherein the first or the second polymerase has 3'-5' exonuclease activity and the polymerase chimera has 5'-3' polymerase activity as well as 3'-5' exonuclease activity. The polymerases can be naturally occurring or recombinant polymerases. The polymerase chimera according to the invention can be composed of functional amino acid fragments from two or several different polymerases. The polymerase chimera according to the invention can be composed of two or several functional amino acid fragments from the different polymerases. The amino acid sequence of the fragment can correspond to the naturally occurring sequence of the polymerase or to a sequence modified by mutations.

The amino acid fragments from which the polymerase

chimera is constructed preferably each correspond to functional polymerase domains of the first or second polymerase. A functional polymerase domain in the sense of the present invention is a region which contains all amino acids that are essential for the activity and is abbreviated as domain in the following.

The present invention concerns in particular a polymerase chimera composed of functional amino acid fragments (in short domains) from at least two different polymerases wherein the domain having polymerase activity is homologous to one polymerase and the domain having 3' exonuclease activity is homologous to another polymerase. Moreover, this chimera can additionally have 5' exonuclease activity in which case the domain having 5' exonuclease activity can be homologous to the first or to the second polymerase. However, it is also possible that the 5' exonuclease domain is partially or completely deleted or has point mutations. The polymerase chimera according to the invention can additionally have reverse transcriptase (RT) activity.

It is additionally preferred that a part of the amino acid fragments of the polymerase chimeras corresponds to a part of the amino acid sequence of Taq polymerase.

The polymerase whose domain or amino acid fragment having 3'-5' exonuclease activity has been incorporated into the chimera can for example be a Pol-I type polymerase or also a Pol-II type polymerase. Representatives of the Pol-I type polymerase with 3'-5' exonuclease activity are for example *Escherichia coli* polymerase (Ec.1), *Salmonella* polymerase I, *Bacillus* polymerase I, *Thermosiphon* polymerase I and *Thermatoga*

neapolitana polymerase (Tne). Representatives of the Pol-II type polymerase with 3'-5' exonuclease activity are for example *Pyrrococcus woesie* polymerase (Pwo), *Pyrococcus furiosus* polymerase (Pfu), *Thermococcus litoralis* polymerase (Tli), *Pyrodictum abyssi*.

Representatives of Pol-I type and Pol-II type polymerases which were mentioned as examples are described in more detail in the following:

The *Taq* DNA polymerase from *Thermus aquaticus* (*Taq* polymerase), *Escherichia coli* DNA polymerase I (E. coli polI) and *Thermotoga neapolitana* DNA polymerase (*Tne* polymerase) are bacterial DNA polymerases from the A family. They are DNA polymerases of the polI type since the various enzymatic activities are located in the various domains in a relatively similar manner to that found in *E. coli* polI. The *Pyrococcus woesi* DNA polymerase (*Pwo* polymerase) is, like *Thermococcus litorales* DNA polymerase (Vent™ polymerase) and *Pyrococcus furiosus* DNA polymerase (*Pfu* polymerase), an archaebacterial DNA polymerase of the B family.

Taq polymerase is described by Chien, A. et al. (1976) J. Bacteriol. 127, 1550-1557, Kaledin, A.S. et al. (1980) Biokhimiya 45, 644-651 and Lawyer, F.C. et al. (1989) J. Biol. Chem. 264, 6427-6437. It was originally isolated from the thermophilic eubacterium *Thermus aquaticus* and later cloned in *E. coli*. The enzyme has a molecular weight of 94 kDa and is active as a monomer. *Taq* polymerase is suitable for use in the polymerase chain reaction (PCR) since it has a high thermal stability (half life of 40 minutes at 95°C/5 minutes at 100°C) and a highly processive 5'-3' DNA polymerase

(polymerisation rate: 75 nucleotides per second). Apart from the polymerase activity, a 5' nuclease activity was detected by Longley et al. (1990) Nucl. Acids Res. 18, 7317-7322. The enzyme has no 3'-5' exonuclease activity so that errors occur during the incorporation of the four deoxyribonucleotide triphosphates to successively extend polynucleotide chains which interfere with the gene amplification (error rate: 2 x 10^{-4} errors/base, Cha, R.S. and Thilly, W.G. (1993) PCR Methods Applic. 3, 18-29). The tertiary structure of *Taq* polymerase has been known since 1995 (Kim et al., 1995, Korolev et al., 1995).

E. coli polI is described in Kornberg, A. and Baker, T.A. (1992) DNA Replication, 2nd edition, Freeman, New York, 113-165. The enzyme has a molecular weight of 103 kDa and is active as a monomer. *E. coli* polI has 5' nuclease activity and 5'-3' DNA polymerase activity. In contrast to *Taq* polymerase, it additionally has a 3'-5' exo-nuclease activity as a proof-reading function. *E. coli* polI and its Klenow fragment (Jacobsen, H. et al. (1974) Eur. J. Biochem. 45, 623-627) were used for PCR before the introduction of *Taq* polymerase. However, due to their low thermal stability they are less suitable since they have to be newly added to each cycle. The tertiary structure of the Klenow fragment of *E. coli* polI has been known since 1983 (Brick, P. et al., (1983) J. Mol. Biol. 166, 453-456, Ollis, D.L. et al. (1985) Nature 313, 762-766 and Beese, L.S. et al. (1993) Science 260, 352-355).

Tne polymerase was isolated from the thermophilic eubacterium *Thermotoga neapolitana* and later cloned in E. coli. The amino acid sequence of the *Tne* polymerase is similar to that of *Thermotoga maritima* DNA polymerase (UITma™ polymerase) (personal information from Dr. B.

Frey). It has a high thermal stability, 5' nuclease activity, 3'-5' exonuclease activity and 5'-3' DNA polymerase activity. A disadvantage is the low polymerisation rate compared with that of *Taq* polymerase. The UITma™ polymerase which has a similar amino acid sequence is used for PCR if a high accuracy is required. Of the structure of *Tne* polymerase, only the amino acid sequence is known up to now (Boehringer Mannheim). However, the enzyme is homologous to *E. coli* polI so that, although the tertiary structure is unknown, homology modelling is possible.

Pfu polymerase was isolated from the hyper-thermophilic, marine archaebacterium *Pyrococcus furiosus*. It has a high thermal stability (95 % activity after one hour at 95°C), 3'-5' exonuclease activity and 5'-3' DNA polymerase activity (Lundberg, K.S. et al. (1991) Gene 108, 1-6). The accuracy of the DNA synthesis is ca. 10 times higher than that of *Taq* polymerase. It is used for PCR if a high accuracy is required. Of the structure only the amino acid sequence is known up to now.

Pwo polymerase (PCR Applications Manual (1995), Boehringer Mannheim GmbH, Biochemica, 28-32) was originally isolated from the hyperthermophilic archae-bacterium *Pyrococcus woesi* and later cloned in *E. coli*. The enzyme has a molecular weight of about 90 kDa and is active as a monomer. *Pwo* polymerase has a higher thermal stability than *Taq* polymerase (half life > 2 hours at 100°C), a highly processive 5'-3' DNA polymerase activity and a high 3'-5' exonuclease activity which increases the accuracy of the DNA synthesis. The enzyme has no 5' nuclease activity. The polymerisation rate (30 nucleotides per second) is less than that of *Taq* polymerase. The enzyme is used for PCR if a high

accuracy is required. The accuracy of the DNA synthesis is more than 10 times higher than when using *Taq* polymerase.

Ath polymerase was isolated from the thermophilic archaebacterium *Anaerocellum thermophilum* and later cloned in *E. coli*. *Ath* polymerase has a high thermal stability and still has at least 90 % of the original activity after an incubation of 30 min at 80°C in the absence of stabilizing detergents. The polymerase also has RT activity in the presence of magnesium ions. *Ath* polymerase is deposited at the "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH", Mascheroder Weg 1b, D38124 Braunschweig DSM Accession No. 8995. The *Ath* polymerase has 5'-3' polymerase activity, 5'-3' exonuclease activity but no 3'-5' exonuclease activity.

Histidine tags or other purification aids can be additionally incorporated into the amino acid sequence of the polymerase chimeras to improve the purification.

There are four main methods for introducing a 3'-5' exonuclease activity of a polymerase into another polymerase for example into *Taq* polymerase which are also a subject matter of the present invention:

1. <u>Insertion of the 3'-5' exonuclease region of another DNA polymerase by exchange of a molecular region of *Taq* polymerase</u>

This approach is particularly suitable since the *Taq* polymerase is homologous to *E. coli* polI which is composed of domains which are functionally and structurally independent (Joyce, C.M. and Steitz, T.A.

(1987) TIBS 12, 288-292) and can serve as a model for other DNA polymerases (Joyce, C.M. (1991) Curr. Opin. Struct. Biol. 1, 123-129). Suitable DNA polymerases for the exchange are those for which a 3'-5' exonuclease activity has been demonstrated, whose DNA sequence is known and the gene coding for the 3'-5' exonuclease activity is available. For a rational protein design based on model structures it is additionally advantageous that the 3'-5' exonuclease region and the polymerase region are homologous to *E. coli* polI. The 3'-5' exonuclease region preferably fits well into the structure of *E. coli* polI and adjoins the polymerase region of *Taq* polymerase. Further advantages are an elucidated tertiary structure with available structural data and high thermal stability of the protein.

The following DNA polymerases are thus for example suitable:

a. *E. coli* polI

Apart from thermal stability, *E. coli* polI fulfils all the above-mentioned conditions. The tertiary structure of the Klenow fragment is available in the Brookhaven data bank and, like *Taq* polymerase, it belongs to the A family of DNA polymerases. The identity in the amino acid sequence is 32 %. Taking the known domain structure into consideration, the largest agreements are found in the N-terminal and in the C-terminal region of the two proteins (32 % identity in the 5' nuclease domains, 49 % identity in the polymerase domains). The shorter *Taq* polymerase has several deletions in the region of the 3'-5' exonuclease domain (14 % identity in the 3'-5' exonuclease domain and intermediate domain). Since *E. coli* polI is thermolabile and the interactions at the interface between the two domains in the chimeric

protein are no longer optimal, it is probable that the protein chimera will also have a lower thermal stability than that of *Taq* polymerase. This can be redressed by subsequent modification of amino acids at the interface.

b. Thermostable DNA polymerases

Among the thermostable DNA polymerases with 3'-5' exonuclease that are nowadays used for PCR, the *Pwo* polymerase, *Pfu* polymerase, Vent™ polymerase, *Tne* polymerase and UITma™ polymerase appear to be suitable for combination with the *Taq* DNA polymerase. The genes of the *Pwo* polymerase and the *Tne* polymerase are accessible (via the Boehringer Mannheim Company). The *Pfu* polymerase can be obtained from Stratagene Inc. The *Tne* polymerase is well suited for a rational protein design due to its homology to *Taq* polymerase and *E. coli* polI. When using the *Pfu* polymerase designs are only possible based on amino acid sequence alignments taking into consideration the known conserved amino acids and motifs that are essential for the function.

2. Modification of the *Taq* DNA polymerase in the intermediate domain

In order to insert a 3'-5' exonuclease activity it is necessary to insert all amino acids that are essential for the activity into the structure. According to the present state of knowledge this applies in particular to the three motifs Exo I, Exo II and Exo III. The essential motifs must additionally be linked in a suitable manner in order to be placed in the spatial position necessary for catalysis.

It is also possible to modify the *Taq* DNA polymerase in the polymerase region. A de novo design of polymerases is also in principle conceivable.

The chimeras according to the invention can be additionally optimized by:

1. Removing the 5' nuclease domain (possible also proteolytically) or subsequently inactivating the 5' nuclease activity (described in Merkens, L.S. (1995) Biochem. Biophys. Acta 1264, 243-248)
2. Modification by point mutations or fragment exchange
3. Optimization of the structures at the interface of the chimeras
4. Optimization by random mutagenesis and/or random recombination with other polymerase genes (molecular evolution).

Examples of polymerase chimeras according to the invention are the following:

- *Taq* DNA polymerase (M1-V307)*E.coli* DNA polymerase (D355-D501) *Taq* DNA polymerase (A406-E832)
- *Taq* DNA polymerase (M1-P291)*E.coli* DNA polymerase (Y327-K511) *Taq* DNA polymerase (L416-E832)
- *Taq* DNA polymerase (M1-P291)*E.coli* DNA polymerase (Y327-H519) *Taq* DNA polymerase (E424-E832): point mutation A643G; Ile455Val SEQ ID NO.:1
- *Taq* DNA polymerase (M1-P291)*E.coli* DNA polymerase (Y327-V536) *Taq* DNA polymerase (L441-E832)
- *Taq* DNA polymerase (M1-P291)*E.coli* DNA polymerase (Y327-G544) *Taq* DNA polymerase (V449-E832); SEQ ID NO.:2
- *Taq* DNA polymerase (M1-P302)*E.coli* DNA polymerase (K348-S365) *Taq* DNA polymerase (A319-E347) *E.coli* DNA

poly(N450-T505) *Taq* DNA polymerase (E410-E4832);

- *Taq* DNA polymerase (M1-V307)*Tne* DNA polymerase (D323-D468) *Taq* DNA polymerase (A406-E832)
- *Taq* DNA polymerase (M1-P291)*Tne* DNA polymerase (P295-I478) *Taq* DNA polymerase (L416-E832)
- *Taq* DNA polymerase (M1-P291)*Tne* DNA polymerase (P295-E485) *Taq* DNA polymerase (E424-E832); silent mutation A1449C SEQ ID NO.:3
- *Taq* DNA polymerase (M1-P291)*Tne* DNA polymerase (P295-V502) *Taq* DNA polymerase (L441-E832)
- *Taq* DNA polymerase (M1-P291)*Tne* DNA polymerase (P295-G510) *Taq* DNA polymerase (V449-E832); silent mutation C1767T SEQ ID NO.:4
- *Taq* DNA polymerase (M1-P302)*Tne* DNA polymerase (E316-D333) *Taq* DNA polymerase (A319-E347) *Tne* DNA polymerase (I381-M394) *Taq* DNA polymerase (R362-L380) *Tne* DNA polymerase (E415-T472)*Taq* DNA polymerase (E410-E832);
- G308D/V310E/L352N/L356D/E401Y/R305D
- *Taq* DNA polymerase (1-291)*Pfu* DNA polymerase (V100-R346) *Taq* DNA polymerase (E424-E832)
- *Taq* DNA polymerase (1-291)*Pfu* DNA polymerase (H103-S334) *Taq* DNA polymerase (E424-E832); SEQ ID NO.:5
- *Taq* DNA polymerase (1-291)*Pfu* DNA polymerase (V100-F389) *Taq* DNA polymerase (E424-E832)
- *Taq* DNA polymerase (1-291)*Pfu* DNA polymerase (V100-F389) *Taq* DNA polymerase (V449-E832); SEQ ID NO.:6
- *Taq* DNA polymerase (1-291)*Pfu* DNA polymerase (M1-F389) *Taq* DNA polymerase (V449-E832)

Of the above-mentioned polymerase chimeras the following were examined in more detail:

- *Taq* DNA polymerase (M1-P291)*E.coli* DNA polymerase (Y327-H519) *Taq* DNA polymerase (E424-E832): point mutation A643G; Ile455Val (Taq Ec1) SEQ ID NO.:1

- *Taq* DNA polymerase (M1-P291)*E.coli* DNA polymerase (Y327-G544) *Taq* DNA polymerase (V449-E832), (Taq Ec2) SEQ ID NO.:2
- *Taq* DNA polymerase (M1-P291)*Tne* DNA polymerase (P295-E485) *Taq* DNA polymerase (E424-E832); silent mutation A1449C (Taq Tne1) SEQ ID NO.:3
- *Taq* DNA polymerase (M1-P291)*Tne* DNA polymerase (P295-G510) *Taq* DNA polymerase (V449-E832); silent mutation C1767T (Taq Tne2) SEQ ID NO.:4
- *Taq* DNA polymerase (1-291)*Pfu* DNA polymerase (V100-R346) *Taq* DNA polymerase (E424-E832), (Taq Pfu1) SEQ ID NO.:5
- *Taq* DNA polymerase (1-291)*Pfu* DNA polymerase (V100-F389) *Taq* DNA polymerase (V449-E832), (Taq Pfu2) SEQ ID NO.:6

In order to select suitable DNA polymerases, multiple amino acid sequence alignments of available sequences of DNA polymerases and DNA binding proteins are established for example with the program GCG (Devereux et al., 1984, Nucl. Acids Res. 12, 387-395). In order to find a good alignment it is necessary to take into consideration the secondary structure predictions, known structure-based sequence alignments, known motifs and functionally essential amino acids as well as phylogenetic aspects. If the proteins are composed of functionally and structurally independent domains it is appropriate to firstly establish the amino acid sequence alignments with respect to the individual domains and only afterwards to combine them into a complete sequence alignment.

If homologous sequences are found whose tertiary structure is known, then it is possible to derive a 3D model structure from the homologous protein. The program BRAGI (Reichelt and Schomburg, 1988, J. Mol. Graph. 6, 161-165) can be used to make the model. The program

AMBER (Weiner et al., 1984, J. Am. Chem. Soc. 106, 765-784) can be used for energy minimization of the structures of individual molecule regions and whole molecules and the program Procheck can be used to check the quality of the model. If only the Cα coordinates of the structure of the initial protein are available, the structure can for example be reconstructed using the program O (Jones et al., 1991, Acta Cryst. A47, 110-119). It is also possible to obtain Cα coordinates that are not available in the protein data bank but have been already published as a stereo picture by scanning the stereo picture and picking out the coordinates (for example using the program Magick) and calculating the z-coordinates (for example using the program stereo). Variants can be designed based on amino acid sequence alignments, based on 3D models or based on experimentally determined 3D structures.

In addition chimera variants were produced in which the domain with polymerase activity has reverse transcriptase activity. Examples of suitable polymerases are e.g. the polymerase from Anaerocellum thermophilum Ath or Thermus thermophilum Tth. The 3'-5' exonuclease activity is inserted by a domain which is derived from another polymerase e.g. the Tne polymerase or the Pfu or Pwo polymerase. This chimera can additionally have 5'-3' exonuclease activity in which case the domain with 5' exonuclease activity can be derived from the first as well as from the second polymerase.

The recombinant hybrid polymerases HYB and HYBd5, like the DNA polymerase from Anaerocellum thermophilum, have a relatively strong reverse transcriptase activity in the presence of magnesium ions as well as in the presence of manganese ions. As shown in Figure 22 the

ratio of polymerase activity to reverse transcriptase activity is more favourable than with the Tth polymerase which is the most common and well-known enzyme of this type. This finding applies to the magnesium-dependent as well as to the manganese-dependent reverse transcriptase activity. It can be concluded from this that the polymerase domain which is derived from the Anaerocellum polymerase also exhibits full activity in the hybrid enzyme. The variant HYBd5 additionally has 3'-5' exonuclease activity as shown in Figure 21. This is inhibited by the presence of deoxynucleoside triphosphates as expected for the typical "proof-reading activity". The exonuclease domain which is derived from the DNA polymerase from Thermotoge neapolitana is thus also active in the hybrid molecule. The ability to inhibit the exonuclease activity also demonstrates that both domains of the hybrid polymerase molecule interact and thus the hybrid polymerase is functionally very similar to the natural enzyme.

The production of domain exchange variants by genetic engineering can be achieved by PCR mutagenesis according to the SOE method (Horton et al. (1989) Gene 77, 61-68) or by the modified method (cf. scheme in the examples) with the aid of chemically synthesized oligodeoxynucleotides. The respective DNA fragments are separated on an agarose gel, isolated and ligated into the starting vector. pUC derivatives with suitable promoters such as pTE, pTaq, pPL, Bluescript can be used as starting vectors for *E. coli*. The plasmid DNA is transformed into an *E. coli* strain, for example XL1-blue, some clones are picked out and their plasmid DNA is isolated. It is also possible to use other strains such as Nova Blue, BL21 (DE), MC1000 etc. Of course it is also possible to clone into other organisms such as

into yeast, plant and mammalian cells. A preselection of clones whose plasmid DNA is sequenced in the modified region is made by restriction analysis.

The gene expression in the target proteins can be induced by IPTG in many plasmids such as Pbtaq. When producing many different variants it is appropriate to establish a universal purification procedure. Affinity chromatography on Ni-NTA (nickel-nitrilotriacetic acid) agarose is well suited for this which can be used after attaching a His tag to the protein, for example by PCR. The protein concentrations can be determined with the protein assay ESL (Boehringer Mannheim) and contaminating side activities of the preparations can be determined as described for the commercially available *Taq* polymerase (Boehringer Mannheim). Polymerase, exonuclease activity and thermostability tests are carried out to further characterize the variants and the respective temperature optimum is determined. The polymerase activities of the chimeras can be determined in non-radioactive test systems for example by determining the incorporation rate of Dig-dUTP into DNase activated calf thymus DNA, or in radioactive test systems by for example determining the incorporation rate of α-[^{32}P]dCTP into M13 mp9 ssDNA. In order to determine the temperature optima of the polymerase activity of the chimeras, the polymerase reaction is carried out at different temperatures and the specific activities are calculated. The residual activities (i.e. the percentage of the initial activity without heat treatment) after heat treatment are measured in order to determine the thermal stabilities. The 3'-5' exonuclease activity can be demonstrated by incorporation of a 5'-Dig-labelled primer which anneals to a DNA template strand starting at its 3' end. The correction of 3'

mismatched primers and their extension (proof reading) can be shown by the extension of mismatched 5'-Dig-labelled primers which anneal to a template strand in the recognition sequence of a restriction enzyme (e.g. EcoRI). A cleavage with the restriction enzyme is only possible when the mismatch is corrected by the enzyme. The processivity can be examined by using variants in the PCR. If the enzyme is not sufficiently thermostable for use in PCR, a PCR can be carried out at the temperature optimum as the extension temperature with successive addition of enzyme. The exonuclease activity of the chimeras can be determined in a radioactive test system. For this a certain amount of the chimeric polymerases (usually 2.5 U) is incubated for 4 hours at various temperatures with labelled DNA (5 μg [^{3}H] DNA in the respective test buffers). dNTPs were optionally added at various concentrations (0 - 0.2 mM). After terminating the reaction the release of radioactively labelled nucleotides is determined.

A further subject matter of the present invention is the DNA sequence of the polymerase chimeras described above. In particular the DNA sequences SEQ ID NO.: 1-6 are a subject matter of the present invention. The present invention additionally concerns the amino acid sequences of the polymerase chimera described above. In particular the amino acid sequences SEQ ID NO.: 7-12 are a subject matter of the present invention. Moreover the DNA sequence SEQ ID NO.:17 is a subject matter of the invention.

Vectors which contain the above-mentioned DNA sequences are a further subject matter of the present invention. pBTaq (plasmid Pbtaq4_oligo 67 (Villbrandt (1995), dissertation, TU Braunschweig)) is a preferred vector.

The *E. coli* strains, in particular the strain Escherichia coli XL1-blue which contain the vector which carries the polymerase chimera gene are a further subject matter of the invention. The following strains were deposited at the DSM, "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH", Mascheroder Weg 1b, D-38124 Braunschweig:

- *E.coli* XL1 Blue x pBTaqEc1: **TaqEc1** DSM No. 12053
- *E.coli* XL1 Blue x pBTaqTne1:**TaqTne1** DSM No. 12050
- *E.coli* XL1 Blue x pBTaqTne2:**TaqTne2** DSM No. 12051
- *E.coli* XL1 Blue x pBTaqPfu1:**TaqPfu1** DSM No. 12052

The polymerase chimeras according to the invention are particularly suitable for amplifying DNA fragments e.g. for the polymerase chain reaction. A further application is for example to sequence DNA fragments.

A preferred vector for the Ath-Tne chimera is the following:
E.coli BL 21 (DE3) plysS x pETHYBR : HYBR
E.coli BL 21 (DE3) plysS x pETHYBR d5: HYBR d5

The E. coli strains which contain the vector which carries the polymerase chimera gene are a further subject matter of the invention. The following strains were deposited at the DSM, "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH", Mascheroder Weg 1b, D-38129 Braunschweig: HYBR (DSM No. 12720); HYBR d5 (DSM No. 12719).

The production of the above-mentioned Ath-Tne chimeras is described for example in examples 8-11. The chimeras according to the invention which have RT activity are

particularly suitable for the reverse transcription of RNA.

A further subject matter of the present invention is a kit for amplifying DNA fragments which contains at least one of the polymerase chimeras according to the invention.

Short description of the Figures

Figure 1:
DNA sequence of the *Taq* DNA polymerase (M1-P291) *E. coli* DNA polymerase (Y327-H519) *Taq* DNA polymerase (E424-E832): point mutation A643G; Ile455Val SEQ ID NO.:1; and the corresponding amino acid sequence SEQ ID NO.:7.

Figure 2:
DNA sequence of the *Taq* DNA polymerase (M1-P291) E. coli DNA polymerase (Y327-G544) *Taq* DNA polymerase (V449-E832); SEQ ID NO.:2; and the corresponding amino acid sequence SEQ ID NO.:8.

Figure 3:
DNA sequence of the *Taq* DNA polymerase (M1-P291) *Tne* DNA polymerase (P295-E485) *Taq* DNA polymerase (E424-E832); silent mutation A1449C SEQ ID NO.:3; and the corresponding amino acid sequence SEQ ID NO.: 9.

Figure 4:
DNA sequence of the *Taq* DNA polymerase (M1-P291) *Tne* DNA polymerase (P295-G510) *Taq* DNA polymerase (V449-E832); silent mutation C1767T SEQ ID NO.:4; and the corresponding amino acid sequence SEQ ID NO.:10.

Figure 5:

DNA sequence of the *Taq* DNA polymerase (1-291) *Pfu* DNA polymerase (H103-S334) *Taq* DNA polymerase (E424-E832); SEQ ID NO.:5; and the corresponding amino acid sequence SEQ ID NO.:11.

Figure 6:

DNA sequence of the *Taq* DNA polymerase (1-291) *Pfu* DNA polymerase (V100-F389) *Taq* DNA polymerase (-V449-E832); SEQ ID NO.:6; and the corresponding amino acid sequence SEQ ID NO.:12.

Figure 7:

Purification of the domain exchange variant TaqEc1 on Ni-NTA agarose. Analysis on an 8 % polyacrylamide gel stained with Coomassie blue.

Lanes: 1,8	protein molecular weight marker Broad Range (200 kDa, 116.25 kDa, 97.4 kDa, 66.2 kDa, 45 kDa, 31 kDa)
lane 2	soluble proteins
lane 3	column flow-through
lane 4	wash fraction buffer B
lane 5	wash fraction buffer A
lanes 6,7	eluate fraction buffer C

protein yield (OD_{280}) about 7 mg

Figure 8:

Determination of protein purity: SDS-PAGE, Phast system (10-15 %): silver staining MW: protein molecular weight markers; NHis-TaqPol: Taq DNA polymerase with N-terminal His tag; TaqEc1, TaqTne1, TaqTne2: domain exchange variants.

Figure 9:
Specific activities of the domain exchange variants at various temperatures.

Figure 10:
Testing the domain exchange variants in the PCR with successive addition of enzyme, extension at 72°C.

lambda DNA (left): size of the target sequence = 500 bp
plasmid pa (right): size of the target sequence = 250 bp
lane 1: *Taq* DNA polymerase (BM Co.), 100 ng, 5 units
lane 2: domain exchange variant TaqEc1, 500 ng, 1.25 units/cycle
lane 3: domain exchange variant TaqTne1, 50 ng, 3.6 units/cycle
lane 4: domain exchange variant TaqTne2, 50 ng, 3.5 units/cycle
III: DNA length standard III (BM Co.)
VI: DNA length standard VI (BM Co.).

Result: When the domain exchange variant TaqTne2 was used, PCR products of the correct size were formed.

Figure 11:
Testing the domain exchange variants in the PCR with successive addition of enzyme, extension at 55°C.

lambda DNA (left): size of the target sequence = 500 bp
plasmid pa (right): size of the target sequence = 250 bp
lane 1: domain exchange variant TaqEc1, 500 ng, 6 units/cycle
lane 2: domain exchange variant TaqTne1, 50 ng, 7.5 units/cycle
III: DNA length standard III (BM Co.)

VI: DNA length standard VI (BM Co.).

Result: When the domain exchange variant TaqEc1 was used, PCR products of the correct size were formed.

Figure 12:
3'-5' exonuclease test-variant TaqEc1, incubation at 72°C, primer P1.

Figure 13:
3'-5' exonuclease test-variant TaqEc1, incubation at 50°C, primer P1 (left), primer P2 (right).

Figure 14:
Correction of 3' mismatched primers and their extension - variant TaqEc1 (3' mismatch primer correction assay)
(-): without restriction enzyme digestion
(+): restriction enzyme digestion with EcoRI.

Figure 15:
Schematic representation
Degradation of primers at the 3' end (3'-5' exonuclease assay) and correction of 3' mismatched primers and their extension (3' mismatch primer correction assay).

Figure 16:
Schematic representation: simplified flow chart, degradation of primers at the 3' end and correction of 3' mismatched primers and extension.

Figure 17:
CLUSTAL W (1.5) multiple sequence alignment of the Ath, Tne, PolI polymerase genes as well as of the predicted

gene of the polymerase chimera. The part of the chimera sequence which is derived from Tne is underlined.

Figure 18:

A. Structure of the primers which were used for the PCR amplification of the Tne-Exo and the Ath polymerase domains.

B. Part of the amino acid sequence alignment of two polymerases which exhibited the selected crossing point.

C. Nucleotide sequence and position of the primers which were designed for the construction of the hybrid polymerase gene. The sequences of the primers which are not complementary to the target sequence are shown in small letters. Complementary "overlapping" sequences in the TNELOW and ATHUP primers are double underlined.

Figure 19:

A. Part of the alignment of the Ath and Tne amino acid sequences which show the homologous region that was used to splice together the domains of the two polymerases.

B. Nucleotide and amino acid sequence of the two polymerases in the splicing region. The figure shows the single BamHI cleavage site in the Tne DNA sequence and the sequence of the two oligos that were constructed in order to introduce the BamHI cleavage site into the Ath polymerase.

Figure 20:
Construction of the gene of the polymerase chimera (cf. also example 8).

Figure 21:
3'-5' exonuclease activity of the recombinant DNA polymerase.

1-DNA of the lambda phage hydrolyzed by HindIII
2-DNA of the lambda phage hydrolyzed by HindIII, and dNTP, and recombinant DNA polymerase
3-DNA of the lambda phage hydrolyzed by HindIII, without dNTP, with recombinant DNA polymerase
4-DNA of the lambda phage hydrolyzed by HindIII.

Figure 22:
Reverse transcriptase activity of the recombinant polymerases HYB and HYBd5. The DNA polymerase activity of a 2 µl extract from E. coli BL21 (DE3) plysS x pETHYBr and E. coli BL21 (DE3) plysS x pETHYBRd5 was determined with a precision of 0.05 units. These amounts were used to determine the reverse transcriptase activity of the hybrid polymerases and the effect of 1 mM manganese or 4 mM magnesium ions. The controls were Tth (0.25 units) as a manganese-dependent reverse transcriptase and C. therm. polymerase (Roche Molecular Biochemicals) as a magnesium-dependent reverse transcriptase.

Example 1: Construction and cloning

Establishing a universal purification procedure
Affinity chromatography on Ni-NTA (nickel-nitrilotri-acetic acid) agarose was used to standardize the

purification protocol for the domain exchange variants. Before producing the protein variants it was necessary to attach or insert a His tag to or into the *Taq* DNA polymerase. Two different His tag variants in the plasmid Pbtaq4_oligo67 (Boehringer Mannheim) were designed and produced. The variant NHis-*Taq*Pol contains an N-terminal His tag, an enterokinase cleavage site to optionally cleave the His tag and an epitope for the detection of His tag proteins with antibodies (Quiagen). It was produced by PCR from the EcoRI site up to the PstI site. In the N-terminal protein sequencing the twenty N-terminal amino acids of the variant NHis-*Taq*Pol were confirmed as correct.

Sequence: NHis-*Taq*Pol

EcoRI codon from TaqPol

5'G AA TTC ATG AGG GGC TCG CAT CAC CAT CAC CAT CAC GCT GCT GAC GAT GAC GAT AAA ATG AGG GGC 3'

Met Arg Gly Ser **His His His His His His** Ala Ala Asp Asp Asp Asp Lys Met Arg Gly

MRGS'His epitope [Met-Arg-Gly-Ser-$(His)_6$] enterokinase [$(Asp)_4$-Lys-X]

SEQ ID No.:13: 5' G AA TTC ATG AGG GGC TCG CAT CAC CAT CAC CAT CAC GCT GCT GAC GAT GAC GAT AAA ATG AGG GGC 3'

SEQ ID No.:14: Met Arg Gly Ser **His His His His His His** Ala Ala Asp Asp Asp Asp Lys Met Arg Gly

The variant 5DHis-*Taq*Pol contains a His tag in a flexible loop of the 5' nuclease domain between glycine 79 and glycine 80 of the *Taq* DNA polymerase and was produced by PCR mutagenesis from the EcoRI site up to the PstI site.

Sequence: 5DHis-*Taq*Pol

SEQ ID No.: 15

SEQ ID No.: 16

5' GAG GCC TAC GGG CAT CAC CAT CAC CAT CAC GGG TAC AAG GCG 3'
GluAlaTyrGly**HisHisHisHisHisHis**GlyTyrLysAla

The correctness of the plasmid DNA in each modified region of the two new genes was confirmed by DNA sequencing. Both modified genes were expressed under the same conditions and at the same rate as the initial protein without a His tag, they could be readily purified by Ni-NTA agarose and behaved like *Taq* polymerase without a His tag in the standard PCR. The N-terminal His tag was used to purify the domain exchange variants.

Amino acid sequence alignments

The following amino acid sequence alignments were set up in order to design the domain exchange variants:

1. *Tne*, *E. coli I* and *Taq* DNA polymerase
2. *Pfu*, *E. coli I* and *Taq* DNA polymerase
3. Multiple amino acid sequence alignments of DNA polymerases

The alignments were established with the program GCG with reference to individual molecule regions (domains) and assembled to form the complete sequence alignment taking into consideration the known secondary structures, motifs and essential amino acids and using the structure-based sequence alignment of the sequences of the 3'-5' exonuclease domain of the Klenow fragment with the corresponding domain of *Taq* DNA polymerase (figure 2d in Kim et al. (1995) Nature 376, 612-616).

In order to select the initial structure of the Klenow fragment for the homology modelling, the structures of

E. coli DNA polymerase I that were available at that time were compared using the program Bragi and an RMS fit:
Klenow fragment-dCMP complex (PDB code: 1dpi), 2.8 Å (1987), Klenow fragment-dCTP complex (PBD-code: 1kfd) 3.9 Å (1993) and Klenow fragment, D355A - DNA complex (PBD-code: 1 kln) 3.2 Å (1994).
The structure Klenow fragment (PDB-code: 1 kln) was selected. Two loops were incorporated into the two regions in which there were no coordinates (Bragi program) and energy-minimized (Amber program). The quality of the protein structure was checked (Procheck program).

Construction of 3D models

A 3D model of the molecular region of the *Taq* DNA polymerase which comprises amino acids 292-832 was constructed using the Bragi program in homology to the structure of the Klenow fragment (PDB-code: 1 kln). The modelling comprised amino acid substitutions, introduction of insertions and deletions, energy-minimization of the new loop regions and energy-minimization of the entire molecule (Amber program).

The structure of *Taq* DNA polymerase was already published at the time of the modelling work but was not available in the protein data bank. In order to set up a model of the intermediate domain of the *Taq* DNA polymerase which corresponds to the 3'-5' exonuclease domain of the Klenow fragment (amino acids 292-423), a stereo picture (Figure 2c in Kim et al. (1995) Nature **376**, 612-616) was scanned, the Cα coordinates were picked out on the screen (x and y coordinates for the

left and right picture) (Magick program, (John Cristy, E.I. du Pont De Nemours and Company Incorporated)), the z coordinates were calculated (Stereo program, (Collaborative Computational Project, Number 4 (1994) Acta Cryst. D50, 760-763)), the protein main chain was reconstructed with generation of a poly-alanine (program O), amino acid substitutions were carried out (Bragi program) and an energy-minimization of the entire molecule was carried out (Amber program). The model of the amino acid residues 292-423 (see above) was added to the model of the polymerase domain (amino acids 424-832) (see above) while allowing for the structural alignments of the *Taq* DNA polymerase with the Klenow fragment (Figure 2b and 2c in Kim et al. (1995) Nature **376**, 612-616). The entire model structure was energy-minimized (Amber program) and the quality of the model structure was checked (Procheck program, (Laskowski, R., A., et al. (1993) J. Appl. Cryst. 26, 283-291)).

A 3D model of the *Tne* DNA polymerase (residues 297-893) was set up in homology to the structure of the Klenow fragment (PDB-code: 1kln). The modelling included amino acid substitutions, introduction of insertions and deletions (Bragi program), energy-minimization of the new loop regions, energy-minimization of the entire molecule (Amber program) and checking the quality of the model structure (Procheck program).

20 Protein variants were designed.
They were based on the 3D structure models when using *E. coli* polI and *Tne* polymerase, and based on the amino acid alignments when using the *Pfu* polymerase.

Production of the domain exchange variants by genetic engineering

The N-terminal His tag was inserted by PCR and the domain exchange variants were produced by a modified SOE method (Horton et al. (1989) Gene 77, 61-68), shown in the scheme with the aid of chemically synthesized oligodeoxynucleotides. The respective DNA fragments were separated on an agarose gel, isolated using the QIAquick gel extraction kit (Qiagen company) according to the protocol supplied and used in PCR reactions I to IV in the subsequent PCR reaction or in the case of the PCR reaction V they were recleaved with the two restriction enzymes whose recognition sequence was located in the flanking primers (EcoRI and Pst I). The ligation of DNA fragments and the production and transformation of competent XL1 Blue *E. coli* cells by electroporation was carried out as described by Villbrandt (1995, Dissertation, TU Braunschweig). Several clones were picked out and their plasmid DNA was isolated according to the protocol supplied using the QIAprep Spin Plasmid Kit (Qiagen company). Microbiological working techniques and the formulations for preparing liquid or plate media as well as the establishment of glycerin cultures was carried out as described in the handbook by Sambrook et al. (1989, Molecular cloning - a laboratory manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The domain exchange variants were expressed at the same rate as the initial protein.

Example 2: Purification (for one chimera)

Purification of the domain exchange variants

All domain exchange variants were isolated by the same protocol from *Escherichia coli* XL1-Blue. The fermentation was carried out for 16 hours at 37°C on a

one litre scale in LB medium/100 mg/ml ampicillin/ 12.5 mg/ml tetraycyclinė/1 mM IPTG. The cells were centrifuged, taken up in 20 ml lysis buffer (50 mM Tris-HCl, pH 8.5, 10 mM 2-mercaptoethanol, 1 mM PMSF), frozen at -70°C for at least 16 hours and treated for 10 minutes with ultrasound. The cell debris was centrifuged and the sterile-filtered supernatant was applied to an Ni-NTA (nickel-nitriloacetic acid) agarose column (Qiagen) with a column volume of 3.5 ml (r=0.65 cm, h=2.7 cm). It was washed with 40 ml buffer A (20 mM Tris-HCl, pH 8.5, 100 mM KCl, 20 mM imidazole, 10 mM 2-mercaptoethanol, 10 % (v/v) glycerol), subsequently with 10 ml buffer B (20 mM Tris-HCl, pH 8.5, 1 M KCl, 20 mM imidazole, 10 mM 2-mercaptoethanol, 10 % (v/v) glycerol) and again with 10 ml buffer A. It was eluted with 15 ml buffer C (20 mM Tris-HCl, pH 8.5, 100 mM KCl, 100 mM imidazole, 10 mM 2-mercaptoethanol, 10 % (v/v) glycerol). The flow rate was 0.5 ml/minute and the fraction size was 10 ml with the wash fractions and 1 ml for the elution fractions. The combined fractions were dialysed against storage buffer (20 mM Tris-HCl pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 % Tween 20, 50 % glycerol) and 200 µg/ml gelatin and Nonidet P40 at a final concentration of 0.5 % were added. The protein solutions were stored at -20°C.

The analysis of the purification of the domain exchange variant TaqEc1 on Ni-NTA agarose is shown in Fig. 7.

Determination of the protein concentration

The protein concentrations were determined by measuring the OD_{280} and with the protein assay ESL (Boehringer Mannheim). Figure 8 shows the determination of the protein purity: SDS-PAGE, Phast system (10-15 %): silver staining.

Example 3: Temperature optimum of the polymerase activity of the chimeras

The polymerase activities of the chimeras were determined in a non-radioactive test system. A radioactive test system was used to adjust the values. The incorporation rate of Dig-dUTP into DN'ase-activated calf thymus DNA was determined in the non-radioactive test system. A 50 µl test mix contained 5 µl buffer mix (500 mM Tris-HCl, 150 mM $(NH_4)_2SO_4$, 100 mM KCl, 70 mM $MgCl_2$, 100 mM 2-mercaptoethanol, pH 8.5), 100 µM each of dATP, dCTP, dGTP, dTTP, 36 nM Dig-dUTP (Boehringer Mannheim), 12 µg calf thymus DNA (DN'ase-activated), 10 µg bovine serum albumin and 2 µl chimeric enzyme or 0.02 units *Taq* polymerase (Boehringer Mannheim) as a reference in dilution buffer (20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 200 µg/ml gelatin, 0.5 % Tween 20, 0.5 % Nonidet P40, 50 % glycerol). The reaction mixtures were incubated for 30 minutes at various temperatures. The reactions were stopped on ice. 5 µl of each reaction mixture was pipetted into white membrane-coated microtitre plates (Pall BioSupport, SM045BWP) and baked for 10 minutes at 70°C. The membrane of the microtitre plate was treated as follows using the accompanying suction trough (Pall Bio Support): apply 100 µl buffer 1 (1 % blocking reagent (Boehringer Mannheim) in 0.1 M maleic acid, 0.15 M NaCl, pH 7.5), incubate for 2 minutes, suck through, repeat once; apply 100 µl buffer 2 (1:10000 diluted anti-Dig-AP-Fab fragment antibodies (Boehringer Mannheim) in buffer 1), incubate for 2 minutes, suck through, repeat once; apply 200 µl buffer 3 (buffer 1 containing 0.3 % Tween 20) under vacuum, repeat once; apply 200 µl buffer 4 (0.1 M Tris-HCl, 0.1 M NaCl, 50 mM $MgCl_2$, pH 9.5) under vacuum; apply 50 µl buffer 5 (1:100 diluted CSPD (Boehringer

Mannheim) in buffer 4), incubate for 5 minutes, suck through. The samples were measured in a luminometer (Microluminar LB 96P, Berthold or Wallac Micro Beta Trilux).

In the radioactive test system the incorporation rate of α-[^{32}P]dCTP into 1 μg M13mp9 ss-DNA was determined. A 50 μl test mix contained 5 μl buffer mix (670 mM Tris-HCl, 50 mM $MgCl_2$, 100 mM 2-mercaptoethanol, 2 % Tesit, 2 mg/ml gelatin, pH 8.8), 10 μM each of dATP, dGTP, dTTP, 5 μM CTP, 0.1 μCi [α-^{32}P]dCTP, 1 μg M13mp9ss DNA annealed with 0.3 μg M13 primer and 1 μl chimeric enzyme or 0.01 units *Taq* polymerase (Boehringer Mannheim) as a reference in dilution buffer (20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 200 μg/ml gelatin, 0.5 % Tween 20, 0.5 % Nonidet P40, 50 % glycerol). In order to prepare the DNA primer mixture, 277.2 μg M13mp9ssDNA (Boehringer Mannheim) and 156 μg M13 sequencing primer (17mer) were heated for 30 minutes to 55°C and cooled for 30 minutes to room temperature. The reaction mixtures were incubated for 30 minutes at 65°C. The reactions were stopped on ice. 25 μl of each of the reaction solutions was removed and pipetted into 250 μl 10 % trichloroacetic acid (TCA)/0.01 M sodium pyrophosphate (PPi), mixed and incubated for 30 minutes on ice. The samples were aspirated over pre-soaked GFC filters (Whatman), the reaction vessels were washed out with 5 % TCA/PPi and the filters were washed at least three times with the same solution. After drying, the filters were measured in a β-counter in 5 ml scintillation liquid. The enzyme samples were diluted in enzyme dilution buffer. 1 μl aliquots of the dilutions were used. Duplicate or triplicate determinations were carried out. The *Taq* DNA polymerase from the Boehringer Mannheim Company was used as a reference.

One unit is defined as the amount of enzyme that is necessary to incorporate 10 nM deoxyribonucleotide triphosphate into acid-precipitatable DNA at 65°C in 30 minutes. In order to determine the standard values, 2 µl aliquots of the total mixture were pipetted onto a dry filter and dried. The blank value was determined by also incubating samples without enzyme and washing them identically.

The temperature optima were determined using the non-radioactive DNA polymerase test at various temperatures.

Specific activities at various temperatures

Enzyme	Temperature [°C]					
	25	37	50	60	72	80
TaqPol (BM)	0.0	0.0	5764.4	8489.1	50000.0	57986.1
NHIs-TaqPol	0.0	0.0	5616.1	12165.2	60843.7	74784.4
TaqEc1	704.9	10353.4	50066.5	41034.4	2677.5	1016.2
TaqTne1	0.0	2559.4	15967.0	18900.4	1100.0	0.0
TaqTne2	747.2	5180.2	23549.6	30627.3	64139.1	28727.4

Example 4: Temperature stability of the polymerase activity of the chimeras

The thermal stability was determined by heating the reaction mixtures to 80°C and 95°C for one, three or six minutes and subsequently determining the residual activities using the non-radioactive DNA polymerase test (see Fig. 9).

Table: residual activities (percent of the initial activity without heat treatment) at 72°C of the Taq DNA polymerase (TaqPol), the *Taq* DNA polymerase with a His

tag (NHis-TaqPol) and the three domain exchange variants (TaqEc1, TaqTne1, TaqTne2) after heat treatment (incorporation of Dig-dUTP into DN'ase-activated calf thymus DNA).

Enzyme	1min 80°C	3 min 80°C	6 min 80°C	1 min 95°C	3 min 95°C	6 min 95°C
TaqPol	100	100	100	100	100	100
NHis-TaqPol	100	100	100	100	100	100
TaqEc1	0	0	0	0	0	0
TaqTne1	16	0	0	0	0	0
TaqTne2	100	100	100	92	0	0

Example 5: PCR with successive addition of enzyme

The polymerase chimeras were tested in a PCR with successive addition of enzyme. The extension was carried out at 72°C (Fig. 10) and at 55°C (Fig. 11). Each of the reactions mixtures with a reaction volume of 100 µl contained 1 ng lambda DNA or pa-plasmid DNA (BM Co.), 1 µM of each primer (25-mer), 200 µM of each of the dNTPs and standard PCR buffer containing $MgCl_2$ (Boehringer Mannheim). The reaction conditions were:

For extension at 72°C: 1 minute 94°C / 30 seconds 50°C / 1 minute 72°C // 25 cycles, 2 minutes at 94°C before and 7 minutes at 72°C after the PCR reaction. 0.5 µl of the domain exchange variants was added per cycle at 50°C.

For extension at 55°C: 1 minute 95°C / 30 seconds 50°C / 1 minute 55°C // 25 cycles, 2 minutes at 95°C before and 7 minutes at 55°C after the PCR reaction. 0.5 µl of the domain exchange variants was added per cycle at 50°C.

Example 6: 3'-5' exonuclease test - TaqEc1 variant

The samples were incubated in the absence of nucleotides with a 5'-Dig-labelled primer which anneals to a DNA template strand. 10 µl test mix contained 1 µl buffer (100 mM Tris-HCl, 15 mM $MgCl_2$, 500 mM KCl, 0.1 mg/ml gelatin, pH 8.3), 1 µl enzyme TaqEc1 (500 units/µl), 1 pmol template strand (50-mer, see scheme) and 500 fmol 5'-Dig-labelled primer P1 (matched, 23mer, see scheme) or P2 (mismatched, 23mer, see scheme). The reaction mixtures were incubated at 50°C for various incubation periods. The DNA fragments were separated on a 12.5 % acrylamide gel (SequaGel Kit, Medco Company) and transferred onto a nylon membrane (Boehringer Mannheim) by contact blotting. The nylon membrane was treated as follows: 100 ml buffer 1 (1 % blocking reagent (Boehringer Mannheim) in 0.1 M maleic acid, 0.15 M NaCl, pH 7.5), incubate for 30 minutes; 100 ml buffer 2 (1:10000 diluted anti-Dig-AP Fab fragment antibody (Boehringer Mannheim) in buffer 1), incubate for 30 minutes; 135 ml buffer 3 each time (buffer 1 containing 0.3 % Tween 20), wash three times for 30 minutes; 50 ml buffer 4 (0.1 M Tris-HCl, 0.1 M NaCl, 50 mM $MgCl_2$, pH 9.5), incubate for 5 minutes; 50 ml buffer 5 (1:1000 diluted CPD star (Boehringer Mannheim) in buffer 4), incubate for 5 minutes. The nylon membrane was dried on Watman paper and exposed for 30 to 60 minutes on a chemiluminescence film (Boehringer Mannheim) for the chemiluminescence detection. If a 3'-5' exonuclease is present, the degradation of the primer at the 3' end is visible (see figures). The *Taq* polymerase with a His tag (NHis-TaqPol) was used as a negative control and the UITma DNA polymerase was used as a positive control. For both control enzymes the reactions mixtures were incubated at 72°C. The reaction buffer of the

manufacturer was used for the UITma DNA polymerase. Fig. 12 and 13 show the 3'-5' exonuclease test variant TaqEc1.

Example 7: Correction of 3'-mismatched primers and their extension - TaqEc1 variant (3'-mismatch primer correction assay)

Dig-labelled primers which anneal to a template strand (50 mer, see scheme) were extended in four different experiments. The primers were a matched primer (P1, 23mer, see scheme) and two different mismatched primers (P2, P3, 23mers, see scheme) which anneal in the recognition sequence of the restriction enzyme EcoRI. A 20 µl test mix contained 1 µl buffer (100 mM Tris-HCl, 15 mM $MgCl_2$, 500 mM KCl, 0.1 mg/ml gelatin, pH 8.3), 1 µl enzyme TaqEc1 (500 units/µl), 10 µM each of dATP, dCTP, dGTP, dTTP, 1 pmol template strand and 500 fmol of each 5'-Dig-labelled primer P1 (matched) or P2 (mismatched) or P3 (mismatched). The reaction mixtures were incubated for 60 minutes at 50°C and afterwards heated for 5 minutes to 95°C. 10 µl aliquots were removed and cleaved for 30 minutes at 37°C with 10 units EcoRI. The DNA fragments were separated on a 12.5 % acrylamide gel (SequaGel Kit, Medco Company) and transferred by contact blot onto a nylon membrane (Boehringer Mannheim). The nylon membrane was treated as described above and exposed for 30 to 60 minutes on a chemiluminescence film (Boehringer Mannheim). When the matched primer was used, the digestion with EcoRI resulted in a 28 bp and a 18 bp fragment. The mismatched primers yield this result only when mismatched nucleotides are replaced by matched nucleotides (see Figure 14).

Example 8: Modification of a recombinant DNA polymerase Design of the hybrid polymerase gene Ath Pol and Tne Pol

Computer prediction

The structure of the chimeric polymerase gene was derived from the sequence alignment (Thompson, J.D. Higgins, D.G. and Gibson, T.J. Nucleic Acids Research, 1994, 22: 4673-4680) between the polymerases and the E. coli POLI gene - the sequence with the highest correspondence with the resolved 3D structure in the data bank of Brookhaven (for the Klenow fragment). The pair alignments showed a correspondence of ca. 40 % and hence the 1KLN structure can presumably be regarded as the best possible prototype. In order to ensure a smooth transition from one structure to the other, the crossing point should be located in an area which has a high similarity with all three proteins from the point of view of multiple alignments. The crossing point should therefore be between the polymerase and 3'-5' exonuclease domain (Figure 17, 18).

Construction of a hybrid polymerase gene and expression vectors.

Computer predictions and simulations serve as a basis for the construction of a hybrid gene. PCR amplification and subcloning were used as methods to obtain the ATH POL and TNE EXO domains in which two primer pairs having the structure shown in Fig. 18 were used. The primers have sequences which are specific for the N- and C-ends of the respective genes and for the connecting sequence in the middle of the gene as shown in Fig. 2B, C. The overlap of 12 bases in the ATHUP and TNELOW primers was designed for the subsequent reconstruction of the hybrid

gene and, furthermore, inserted in an unequivocal SalI restriction site which can be used for further modifications with polymerase domains. The overhangs of the 5' sequence of the TNEUP and ATHLOW primers code for the restriction sites NcoI and HindIII for the later subcloning of the required fragments in the expression vector.

However, applying this strategy requires extensive sequencing of the subcloned regions. For this reason an additional construct was built and the splice connection between the genes was moved to another position i.e. 42 amino acids further below the original connecting position to a region between the polymerases which has a higher similarity. An advantage of the new design is the unequivocal BamHI sequence within the TNE polymerase sequence containing the proposed splice connection. In order to construct the hybrid gene, a BamHI sequence was incorporated into the ATH polymerase sequence which is subsequently used to assemble parts of the gene by a directed mutagenesis. The amino acids and nucleotide sequence of the new compound is shown in Fig. 19.

The hybrid polymerase gene was constructed as described in Fig. 20 by multiple subcloning, directed mutagenesis and sequencing steps.

All fragments obtained by the PCR amplification were sequenced starting at the ends up to the unequivocal restriction sites used in the subsequent subcloning steps. In order to ensure the accuracy of the amplification the PCR reactions were carried out with Vent polymerase (New England Biolabs). The directed mutagenesis was carried out using the "Quick Change"

method (Stratagene).

Example 9: Expression of a hybrid polymerase gene in E. coli

The plasmids pETHYBR and pETHYBRd5 were transformed in the E. coli strain BL21 (DE3) plySS from Novagene and led to the expression of T7 polymerase.

The expression of the hybrid POL gene was monitored in the extracts of recombinant strains by measuring the DNA polymerase activity using the activated DNA assay. The following conditions were used.

1) The recombinant E. coli strains were cultured in LB medium containing 100 mcg/ml ampicillin + 30 mcg/ml chloroamphenicol (for pETHYBR and pETHYBRd5 in BL21 (DE3) plysS) or in 20 ml LB medium containing 100 mcg/ml ampicillin + 30 mcg/ml kanamycin (pARHYBd5 in JM109/pSB1611).

2) The cultures were shaken at 37°C to an optical density of OD 550 ~ 0.6-0.7; then the cultures were cooled to 25° - 28°C, IPTG was added to a final concentration of 1 mM. The incubation was then continued at 25-30°C: For two pET vectors the density of non-induced cultures after 4 hours incubation was OD 550 ~ 2.2 and for induced cultures ~ 1.5.

3) Protein extracts of BL21 (DE3) plysS strains were produced by pelleting 5 ml aliquots of the cultures; the cell pellets were then resuspended in 100 µl termination buffer containing 40 mM Tris-HCl, pH 8.0,

0.1 mM EDTA, 7 mM 2-mercaptoethanol, 0.2 mM PMSF, 0.1 % Triton X-100. The cell extracts were prepared by freezing and thawing the cell suspension in two cycles in liquid nitrogen/warm water bath; then a KCl solution was added to a final concentration of 0.75 M and the extracts of the induced and non-induced cultures were heated for 15 min at 72°C, pelleted and used to measure the polymerase activity; this was carried out in an activated DNA assay (100 mcg/ml activated DNA, 3 mM $MgSO_4$, 50 mM Tris-HCl, pH 8.9, 0.1 % Triton X-100, 70 µM dA-P33, 5-10 µCi/ml) in a volume of 20 µl using 2 µl heated cell extracts.

The results are shown in the following table:

Relative DNA polymerase activity in extracts of recombinant strains (% incorporation of labels, mean of 3 independent measurements)

Strain	BL21 (DE3) plyS			
plasmid	pETHYBR		pETHYBRd5	
IPTG	-	+	-	+
TCA insoluble r/a	5	40	2	85

These data show that both versions of the hybrid polymerase gene could be expressed with the pET vector system.

Characterization of the recombinant hybrid polymerase.

Thermal stability

The thermal stability of recombinant polymerases was determined by heating the extract of the E. coli strain for various periods (10, 30, 60, 120 minutes) at 95°C. It turned out that the completely formed as well as the shortened hybrid polymerase were not sufficiently stable (100 % inactive after a 10 minute incubation at 95°C). The degree of expression of the recombinant polymerases was evaluated by analysing the heated cell extracts in 10 % SDS PAAG; since no visible difference was found between the induced and non-induced cultures, it may be concluded that the production of hybrid polymerases does not exceed 1 % of the total soluble protein.

Proof-reading activity

The proof-reading activity of the recombinant DNA polymerase derived from pETHYBRd5, e.g. Klenow fragment was tested according to the same protocol which was also used for the archaeal DNA. It turned out that the recombinant enzyme has proof reading activity.

Reverse transcriptase activity

The following reaction mixture was used to determine the reverse transcriptase activity: 1µg polydA-$(dT)_{15}$, 330 µM TTP, 0.36 µM digoxigenin-dUTP, 200 µg/ml BSA, 10 mM Tris HCl, pH 8.5, 20 mM KCl. The concentration of $MgCl_2$ in the reaction mixture varied between 0.5 and 10 mM. DTE was added at a concentration of 10 mM.

2 µl recombinant DNA polymerase (derived from pETHYBRd5, e.g. Klenow fragment) was added to the reaction mixture and incubated for 15 min at 50°C. Tth DNA polymerase containing Mn^{2+} was added as a positive control. After stopping the reaction, the mixture was applied to a positively charged nylon membrane (BM). The incorporated digoxigenin was detected by means of the BM protocol, 1995.

It turned out that the recombinant enzymes (Klenow fragment) have reverse transcriptase activity (Fig. 22). The activity is dependent on the presence of Mn^{2+} (optimal concentration 1 mM). The presence of Mg^{2+} had moreover an additional stimulating effect (optimal Mg^{2+} concentration 4 mM).

Example 11: Construction of the chimeric polymerase gene (see Fig. 20)

Abbreviations for the restriction sequences - B-BamHI, Bsp-BspHI, H-HindIII, N-NcoI, R-EcoRI, S-SalI, Sn-SnaI, X-XhoI, Xm-XmaI

1. PCR amplification of the ATH POL domain using the primers ATH UP and ATHLOW and the pARHis10 plasmid containing the complete polymerase gene in the vector pTrcHISB and subcloning in the pSK+Bluescript plasmid → pBSAT. The insertion was sequenced from the flanking primers and it turned out that due to an error during the primer synthesis, a single base in the ATHUP primer sequence had been deleted.

2. Directed mutagenesis of the plasmid pARHis10 with primers m1 and m2 using the "Quick change" procedure

(Stratagene) to incorporate the BamHI sequence at position 1535 → pARHis10mut.

3. PCR amplification of the TNE EXO domain using the primers TNEUP and TNELOW on the template of the pTNEC2 plasmid and subcloning in the SmaI cut puC19 plasmid → pTEX1 and pTEX2 with different orientations of the incorporation.

3. Subcloning the 1444 bp XhoI-BamHI fragment from the pTNEC2 plasmid containing the "LONG" EXO domain in the XhoI-BamHI cut plasmid pTEX1 → pTEXL.

5. Incorporation of the complete ATH polymerase gene as a 2553 bp BamHI-HindII fragment in BamHI-HindIII cut pTEXL → pTEXLATF.

6. Substitution of the XmaI-SnaI fragment of the pTEXLATF plasmid by the 1094 bp XmaI-SnaI fragment from the pARHis10mut plasmid containing the incorporated BamHI sequence → pTEXLATF*.

7. Incorporation of the 4214 bp NcoIHindII fragment from pTEXLATF* into the NcoI-HindII cut pET21d vector → pETNAT.

8. Deletion of the 1535 bp BamHI fragment coding for the N-terminal domain of the ATH polymerase from the pETNAT plasmid; this leads to an in-frame joining of the TNE EXOL and ATH POL sequences → pETHYBR.

9. Substitution of the 1661 bp NcoI-BamHI fragment of pETHYBR by the 829 bp BspHI-BamHI fragment from pETNAT; this leads to the use of Met284 of the TNE polymerase as the starting codon and to deletion of the N-terminal domain with the assumed 5'-3' exonuclease activity → pETHYBRd5.

Claims

1. Polymerase chimera composed of functional amino acid fragments of at least two different polymerases, wherein the functional amino acid fragments are active in the polymerase chimera and the domain having polymerase activity is derived from the first polymerase and the domain having 3'-5' exonuclease activity is derived from the second polymerase and wherein the amino acid sequence of the polymerase chimera essentially corresponds to SEQ ID NO:8.

2. Polymerase chimera composed of functional amino acid fragments of at least two different polymerases, wherein the functional amino acid fragments are active in the polymerase chimera and the domain having polymerase activity is derived from the first polymerase and the domain having 3'-5' exonuclease activity is derived from the second polymerase and wherein the amino acid sequence of the polymerase chimera essentially corresponds to SEQ ID NO:10.

3. Polymerase chimera composed of functional amino acid fragments of at least two different polymerases, wherein the functional amino acid fragments are active in the polymerase chimera and the domain having polymerase activity is derived from the first polymerase and the domain having 3'-5' exonuclease activity is derived from the second polymerase and wherein the amino acid sequence of the polymerase chimera essentially corresponds to SEQ ID NO:12.

4. Polymerase chimera as claimed in one of the claims 1-3, wherein the chimera additionally has RT activity.

5. Polymerase chimera as claimed in one of the claims 1-4, wherein histine tags have been incorporated into the amino acid sequence of the chimera.

6. DNA sequence of a polymerase chimera as claimed in one of the claims 1-5.

7. DNA sequence of a polymerase chimera according to SEQ ID NO.2.

8. DNA sequence of a polymerase chimera according to SEQ ID NO.4.

9. DNA sequence of a polymerase chimera according to SEQ ID NO.6.

10. Vector containing a DNA sequence as claimed in claims 6-9.

11. Transformed cell which contains the vector as claimed in claim 10.

12. Process for the production of the polymerase chimeras as claimed in one of the claims 1-5, wherein the process comprises the following steps:

- designing variants with the aid of amino acid sequence alignments, of 3D models or with the aid of experimentally determined 3D structures

- production of domain exchange variants by genetic engineering
- ligating the DNA fragments into starting vectors
- expression of the chimeras in a host which was transformed by vectors carrying DNA fragments
- purifying the expressed polymerase chimeras.

13. Use of the polymerase chimeras as claimed in one of the claims 1-5 for PCR.

14. Use of the polymerase chimeras as claimed in one of the claims 1-5 to sequence DNA fragments.

15. Use of the polymerase chimeras as claimed in one of the claims 1-5 for RT-PCR starting with an RNA template.

16. Kit containing a polymerase chimera as claimed in one of the claims 1-5.

Abstract

The invention concerns polymerase chimeras which are composed of amino acid fragments representing domains and which combine properties of naturally occurring polymerases that are advantageous with regard to a particular application. It has surprisingly turned out that the domains from the various enzymes are active in the chimeras and exhibit cooperative behaviour. In addition the present invention concerns a process for the production of the chimeras according to the invention and the use of these chimeras for the synthesis of nucleic acids e.g. during a polymerase chain reaction. Moreover the present invention concerns a kit which contains the polymerase chimeras according to the invention.

Figure 1/1
SEQ ID No.: 1

DNA sequence:

```
   1 ATGAGGGGCT CGCATCACCA TCACCATCAC GCTGCTGACG ATGACGATAA
  51 AATGAGGGGC ATGCTACCGC TATTTGAGCC CAAGGGCCGG GTCCTCCTGG
 101 TCGACGGCCA CCACCTGGCC TACCGCACCT TCCACGCCCT GAAGGGCCTC
 151 ACCACCAGCC GGGGGGAGCC GGTGCAGGCG GTCTACGGCT TCGCCAAGAG
 201 CCTCCTCAAG GCCCTCAAGG AGGACGGGGA CGCGGTGATC GTGGTCTTTG
 251 ACGCCAAGGC CCCCTCCTTC CGCCACGAGG CCTACGGGGG GTACAAGGCG
 301 GGCCGGGCCC CCACGCCGGA GGACTTTCCC CGGCAACTCG CCCTCATCAA
 351 GGAGCTGGTG GACCTCCTGG GGCTGGCGCG CCTCGAGGTC CCGGGCTACG
 401 AGGCGGACGA CGTCCTGGCC AGCCTGGCCA AGAAGGCGGA AAAGGAGGGC
 451 TACGAGGTCC GCATCCTCAC CGCCGACAAA GACCTTTACC AGCTCCTTTC
 501 CGACCGCATC CACGTCCTCC ACCCCGAGGG GTACCTCATC ACCCCGGCCT
 551 GGCTTTGGGA AAAGTACGGC CTGAGGCCCG ACCAGTGGGC CGACTACCGG
 601 GCCCTGACCG GGGACGAGTC CGACAACCTT CCCGGGGTCA AGGGCATCGG
 651 GGAGAAGACG GCGAGGAAGC TTCTGGAGGA GTGGGGGAGC CTGGAAGCCC
 701 TCCTCAAGAA CCTGGACCGG CTGAAGCCCG CCATCCGGGA GAAGATCCTG
 751 GCCCACATGG ACGATCTGAA GCTCTCCTGG GACCTGGCCA AGGTGCGCAC
 801 CGACCTGCCC CTGGAGGTGG ACTTCGCCAA AAGGCGGGAG CCCGACCGGG
 851 AGAGGCTTAG GGCCTTTCTG GAGAGGCTTG AGTTTGGCAG CCTCCTCCAC
 901 GAGTTCGGCC TTCTGGAAAG CCCCTATGAC AACTACGTCA CCATCCTTGA
 951 TGAAGAAACA CTGAAAGCGT GGATTGCGAA GCTGGAAAAA GCGCCGGTAT
1001 TTGCATTTGA TACCGAAACC GACAGCCTTG ATAACATCTC TGCTAACCTG
1051 GTCGGGCTTT CTTTTGCTAT CGAGCCAGGC GTAGCGGCAT ATATTCCGGT
1101 TGCTCATGAT TATCTTGATG CGCCCGATCA AATCTCTCGC GAGCGTGCAC
1151 TCGAGTTGCT AAAACCGCTG CTGGAAGATG AAAAGGCGCT GAAGGTCGGG
1201 CAAAACCTGA AATACGATCG CGGTATTCTG GCGAACTACG GCATTGAACT
1251 GCGTGGGATT GCGTTTGATA CCATGCTGGA GTCCTACATT CTCAATAGCG
1301 TTGCCGGGCG TCACGATATG GACAGCCTCG CGGAACGTTG GTTGAAGCAC
1351 AAAACCATCA CTTTTGAAGA GATTGCTGGT AAAGGCAAAA ATCAACTGAC
1401 CTTTAACCAG ATTGCCCTCG AAGAAGCCGG ACGTTACGCC GCCGAAGATG
1451 CAGATGTCAC CTTGCAGTTG CATCTGAAAA TGTGGCCGGA TCTGCAAAAA
1501 CACGAGAGGC TCCTTTGGCT TTACCGGGAG GTGGAGAGGC CCCTTTCCGC
1551 TGTCCTGGCC CACATGGAGG CCACGGGGGT GCGCCTGGAC GTGGCCTATC
1601 TCAGGGCCTT GTCCCTGGAG GTGGCCGAGG AGGTCGCCCG CCTCGAGGCC
1651 GAGGTCTTCC GCCTGGCCGG CCACCCCTTC AACCTCAACT CCCGGGACCA
1701 GCTGGAAAGG GTCCTCTTTG ACGAGCTAGG GCTTCCCGCC ATCGGCAAGA
1751 CGGAGAAGAC CGGCAAGCGC TCCACCAGCG CCGCCGTCCT GGAGGCCCTC
1801 CGCGAGGCCC ACCCCATCGT GGAGAAGATC CTGCAGTACC GGGAGCTCAC
1851 CAAGCTGAAG AGCACCTACA TTGACCCCTT GCCGGACCTC ATCCACCCCA
1901 GGACGGGCCG CCTCCACACC CGCTTCAACC AGACGGCCAC GGCCACGGGC
1951 AGGCTAAGTA GCTCCGATCC CAACCTCCAG AACATCCCCG TCCGCACCCC
2001 GCTTGGGCAG AGGATCCGCC GGGCCTTCAT CGCCGAGGAG GGGTGGCTAT
2051 TGGTGGCCCT GGACTATAGC CAGATAGAGC TCAGGGTGCT GGCCCACCTC
2101 TCCGGCGACG AGAACCTGAT CCGGGTCTTC CAGGAGGGGC GGGACATCCA
2151 CACGGAGACC GCCAGCTGGA TGTTCGGCGT CCCCCGGGAG GCCGTGGACC
2201 CCCTGATGCG CCGGGCGGCC AAGACCATCA ACTTCGGGGT CCTCTACGGC
2251 ATGTCGGCCC ACCGCCTCTC CCAGGAGCTA GCCATCCCTT ACGAGGAGGC
2301 CCAGGCCTTC ATTGAGCGCT ACTTTCAGAG CTTCCCCAAG GTGCGGGCCT
2351 GGATTGAGAA GACCCTGGAG GAGGGCAGGA GGCGGGGGTA CGTGGAGACC
2401 CTCTTCGGCC GCCGCCGCTA CGTGCCAGAC CTAGAGGCCC GGGTGAAGAG
2451 CGTGCGGGAG GCGGCCGAGC GCATGGCCTT CAACATGCCC GTCCAGGGCA
2501 CCGCCGCCGA CCTCATGAAG CTGGCTATGG TGAAGCTCTT CCCCAGGCTG
```

Figure 1/2
SEQ ID No.: 1

```
2551  GAGGAAATGG GGGCCAGGAT GCTCCTTCAG GTCCACGACG AGCTGGTCCT
2601  CGAGGCCCCA AAAGAGAGGG CGGAGGCCGT GGCCCGGCTG GCCAAGGAGG
2651  TCATGGAGGG GGTGTATCCC CTGGCCGTGC CCCTGGAGGT GGAGGTGGGG
2701  ATAGGGGAGG ACTGGCTCTC CGCCAAGGAG TGA
```

SEQ ID No.: 7

amino acid sequence:

```
   1  MRGSHHHHHH AADDDDKMRG MLPLFEPKGR VLLVDGHHLA YRTFHALKGL
  51  TTSRGEPVQA VYGFAKSLLK ALKEDGDAVI VVFDAKAPSF RHEAYGGYKA
 101  GRAPTPEDFP RQLALIKELV DLLGLARLEV PGYEADDVLA SLAKKAEKEG
 151  YEVRILTADK DLYQLLSDRI HVLHPEGYLI TPAWLWEKYG LRPDQWADYR
 201  ALTGDESDNL PGVKGIGEKT ARKLLEEWGS LEALLKNLDR LKPAIREKIL
 251  AHMDDLKLSW DLAKVRTDLP LEVDFAKRRE PDRERLRAFL ERLEFGSLLH
 301  EFGLLESPYD NYVTILDEET LKAWIAKLEK APVFAFDTET DSLDNISANL
 351  VGLSFAIEPG VAAYIPVAHD YLDAPDQISR ERALELLKPL LEDEKALKVG
 401  QNLKYDRGIL ANYGIELRGI AFDTMLESYI LNSVAGRHDM DSLAERWLKH
 451  KTITFEEIAG KGKNQLTFNQ IALEEAGRYA AEDADVTLQL HLKMWPDLQK
 501  HERLLWLYRE VERPLSAVLA HMEATGVRLD VAYLRALSLE VAEEVARLEA
 551  EVFRLAGHPF NLNSRDQLER VLFDELGLPA IGKTEKTGKR STSAAVLEAL
 601  REAHPIVEKI LQYRELTKLK STYIDPLPDL IHPRTGRLHT RFNQTATATG
 651  RLSSSDPNLQ NIPVRTPLGQ RIRRAFIAEE GWLLVALDYS QIELRVLAHL
 701  SGDENLIRVF QEGRDIHTET ASWMFGVPRE AVDPLMRRAA KTINFGVLYG
 751  MSAHRLSQEL AIPYEEAQAF IERYFQSFPK VRAWIEKTLE EGRRRGYVET
 801  LFGRRRYVPD LEARVKSVRE AAERMAFNMP VQGTAADLMK LAMVKLFPRL
 851  EEMGARMLLQ VHDELVLEAP KERAEAVARL AKEVMEGVYP LAVPLEVEVG
 901  IGEDWLSAKE
```

Figure 2/1
SEQ ID No.: 2

DNA sequence:

```
   1 ATGAGGGGCT CGCATCACCA TCACCATCAC GCTGCTGACG ATGACGATAA
  51 AATGAGGGGC ATGCTACCGC TATTTGAGCC CAAGGGCCGG GTCCTCCTGG
 101 TCGACGGCCA CCACCTGGCC TACCGCACCT TCCACGCCCT GAAGGGCCTC
 151 ACCACCAGCC GGGGGGAGCC GGTGCAGGCG GTCTACGGCT TCGCCAAGAG
 201 CCTCCTCAAG GCCCTCAAGG AGGACGGGGA CGCGGTGATC GTGGTCTTTG
 251 ACGCCAAGGC CCCCTCCTTC CGCCACGAGG CCTACGGGGG GTACAAGGCG
 301 GGCCGGGCCC CCACGCCGGA GGACTTTCCC CGGCAACTCG CCCTCATCAA
 351 GGAGCTGGTG GACCTCCTGG GGCTGGCGCG CCTCGAGGTC CCGGGCTACG
 401 AGGCGGACGA CGTCCTGGCC AGCCTGGCCA AGAAGGCGGA AAAGGAGGGC
 451 TACGAGGTCC GCATCCTCAC CGCCGACAAA GACCTTTACC AGCTCCTTTC
 501 CGACCGCATC CACGTCCTCC ACCCCGAGGG GTACCTCATC ACCCCGGCCT
 551 GGCTTTGGGA AAAGTACGGC CTGAGGCCCG ACCAGTGGGC CGACTACCGG
 601 GCCCTGACCG GGGACGAGTC CGACAACCTT CCCGGGGTCA AGGGCATCGG
 651 GGAGAAGACG GCGAGGAAGC TTCTGGAGGA GTGGGGGAGC CTGGAAGCCC
 701 TCCTCAAGAA CCTGGACCGG CTGAAGCCCG CCATCCGGGA GAAGATCCTG
 751 GCCCACATGG ACGATCTGAA GCTCTCCTGG GACCTGGCCA AGGTGCGCAC
 801 CGACCTGCCC CTGGAGGTGG ACTTCGCCAA AAGGCGGGAG CCCGACCGGG
 851 AGAGGCTTAG GGCCTTTCTG GAGAGGCTTG AGTTTGGCAG CCTCCTCCAC
 901 GAGTTCGGCC TTCTGGAAAG CCCCTATGAC AACTACGTCA CCATCCTTGA
 951 TGAAGAAACA CTGAAAGCGT GGATTGCGAA GCTGGAAAAA GCGCCGGTAT
1001 TTGCATTTGA TACCGAAACC GACAGCCTTG ATAACATCTC TGCTAACCTG
1051 GTCGGGCTTT CTTTTGCTAT CGAGCCAGGC GTAGCGGCAT ATATTCCGGT
1101 TGCTCATGAT TATCTTGATG CGCCCGATCA AATCTCTCGC GAGCGTGCAC
1151 TCGAGTTGCT AAAACCGCTG CTGGAAGATG AAAAGGCGCT GAAGGTCGGG
1201 CAAAACCTGA AATACGATCG CGGTATTCTG GCGAACTACG GCATTGAACT
1251 GCGTGGGATT GCGTTTGATA CCATGCTGGA GTCCTACATT CTCAATAGCG
1301 TTGCCGGGCG TCACGATATG GACAGCCTCG CGGAACGTTG GTTGAAGCAC
1351 AAAACCATCA CTTTTGAAGA GATTGCTGGT AAAGGCAAAA ATCAACTGAC
1401 CTTTAACCAG ATTGCCCTCG AAGAAGCCGG ACGTTACGCC GCCGAAGATG
1451 CAGATGTCAC CTTGCAGTTG CATCTGAAAA TGTGGCCGGA TCTGCAAAAA
1501 CACAAAGGGC CGTTGAACGT CTTCGAGAAT ATCGAAATGC CGCTGGTGCC
1551 GGTGCTTTCA CGCATTGAAC GTAACGGTGT GCGCCTGGAC GTGGCCTATC
1601 TCAGGGCCTT GTCCCTGGAG GTGGCCGAGG AGATCGCCCG CCTCGAGGCC
1651 GAGGTCTTCC GCCTGGCCGG CCACCCCTTC AACCTCAACT CCCGGGACCA
1701 GCTGGAAAGG GTCCTCTTTG ACGAGCTAGG GCTTCCCGCC ATCGGCAAGA
1751 CGGAGAAGAC CGGCAAGCGC TCCACCAGCG CCGCCGTCCT GGAGGCCCTC
1801 CGCGAGGCCC ACCCCATCGT GGAGAAGATC CTGCAGTACC GGGAGCTCAC
1851 CAAGCTGAAG AGCACCTACA TTGACCCCTT GCCGGACCTC ATCCACCCCA
1901 GGACGGGCCG CCTCCACACC CGCTTCAACC AGACGGCCAC GGCCACGGGC
1951 AGGCTAAGTA GCTCCGATCC CAACCTCCAG AACATCCCCG TCCGCACCCC
2001 GCTTGGGCAG AGGATCCGCC GGGCCTTCAT CGCCGAGGAG GGGTGGCTAT
2051 TGGTGGCCCT GGACTATAGC CAGATAGAGC TCAGGGTGCT GGCCCACCTC
2101 TCCGGCGACG AGAACCTGAT CCGGGTCTTC CAGGAGGGGC GGGACATCCA
2151 CACGGAGACC GCCAGCTGGA TGTTCGGCGT CCCCCGGGAG GCCGTGGACC
2201 CCCTGATGCG CCGGGCGGCC AAGACCATCA ACTTCGGGGT CCTCTACGGC
2251 ATGTCGGCCC ACCGCCTCTC CCAGGAGCTA GCCATCCCTT ACGAGGAGGC
2301 CCAGGCCTTC ATTGAGCGCT ACTTTCAGAG CTTCCCCAAG GTGCGGGCCT
2351 GGATTGAGAA GACCCTGGAG GAGGGCAGGA GGCGGGGGTA CGTGGAGACC
2401 CTCTTCGGCC GCCGCCGCTA CGTGCCAGAC CTAGAGGCCC GGGTGAAGAG
2451 CGTGCGGGAG GCGGCCGAGC GCATGGCCTT CAACATGCCC GTCCAGGGCA
2501 CCGCCGCCGA CCTCATGAAG CTGGCTATGG TGAAGCTCTT CCCCAGGCTG
```

Figure 2/2
SEQ ID No.: 2

```
2551  GAGGAAATGG GGGCCAGGAT GCTCCTTCAG GTCCACGACG AGCTGGTCCT
2601  CGAGGCCCCA AAAGAGAGGG CGGAGGCCGT GGCCCGGCTG GCCAAGGAGG
2651  TCATGGAGGG GGTGTATCCC CTGGCCGTGC CCCTGGAGGT GGAGGTGGGG
2701  ATAGGGGAGG ACTGGCTCTC CGCCAAGGAG TGA
```

SEQ ID No.: 8

amino acid sequence:

```
  1   MRGSHHHHHH AADDDDKMRG MLPLFEPKGR VLLVDGHHLA YRTFHALKGL
 51   TTSRGEPVQA VYGFAKSLLK ALKEDGDAVI VVFDAKAPSF RHEAYGGYKA
101   GRAPTPEDFP RQLALIKELV DLLGLARLEV PGYEADDVLA SLAKKAEKEG
151   YEVRILTADK DLYQLLSDRI HVLHPEGYLI TPAWLWEKYG LRPDQWADYR
201   ALTGDESDNL PGVKGIGEKT ARKLLEEWGS LEALLKNLDR LKPAIREKIL
251   AHMDDLKLSW DLAKVRTDLP LEVDFAKRRE PDRERLRAFL ERLEFGSLLH
301   EFGLLESPYD NYVTILDEET LKAWIAKLEK APVFAFDTET DSLDNISANL
351   VGLSFAIEPG VAAYIPVAHD YLDAPDQISR ERALELLKPL LEDEKALKVG
401   QNLKYDRGIL ANYGIELRGI AFDTMLESYI LNSVAGRHDM DSLAERWLKH
451   KTITFEEIAG KGKNQLTFNQ IALEEAGRYA AEDADVTLQL HLKMWPDLQK
501   HKGPLNVFEN IEMPLVPVLS RIERNGVRLD VAYLRALSLE VAEEIARLEA
551   EVFRLAGHPF NLNSRDQLER VLFDELGLPA IGKTEKTGKR STSAAVLEAL
601   REAHPIVEKI LQYRELTKLK STYIDPLPDL IHPRTGRLHT RFNQTATATG
651   RLSSSDPNLQ NIPVRTPLGQ RIRRAFIAEE GWLLVALDYS QIELRVLAHL
701   SGDENLIRVF QEGRDIHTET ASWMFGVPRE AVDPLMRRAA KTINFGVLYG
751   MSAHRLSQEL AIPYEEAQAF IERYFQSFPK VRAWIEKTLE EGRRRGYVET
801   LFGRRRYVPD LEARVKSVRE AAERMAFNMP VQGTAADLMK LAMVKLFPRL
851   EEMGARMLLQ VHDELVLEAP KERAEAVARL AKEVMEGVYP LAVPLEVEVG
901   IGEDWLSAKE
```

Figure 3/1
SEQ ID No.: 3

DNA sequence:

```
   1 ATGAGGGGCT CGCATCACCA TCACCATCAC GCTGCTGACG ATGACGATAA
  51 AATGAGGGGC ATGCTACCGC TATTTGAGCC CAAGGGCCGG GTCCTCCTGG
 101 TCGACGGCCA CCACCTGGCC TACCGCACCT TCCACGCCCT GAAGGGCCTC
 151 ACCACCAGCC GGGGGGAGCC GGTGCAGGCG GTCTACGGCT TCGCCAAGAG
 201 CCTCCTCAAG GCCCTCAAGG AGGACGGGGA CGCGGTGATC GTGGTCTTTG
 251 ACGCCAAGGC CCCCTCCTTC CGCCACGAGG CCTACGGGGG GTACAAGGCG
 301 GGCCGGGCCC CCACGCCGGA GGACTTTCCC CGGCAACTCG CCCTCATCAA
 351 GGAGCTGGTG GACCTCCTGG GGCTGGCGCG CCTCGAGGTC CCGGGCTACG
 401 AGGCGGACGA CGTCCTGGCC AGCCTGGCCA AGAAGGCGGA AAAGGAGGGC
 451 TACGAGGTCC GCATCCTCAC CGCCGACAAA GACCTTTACC AGCTCCTTTC
 501 CGACCGCATC CACGTCCTCC ACCCCGAGGG GTACCTCATC ACCCCGGCCT
 551 GGCTTTGGGA AAAGTACGGC CTGAGGCCCG ACCAGTGGGC CGACTACCGG
 601 GCCCTGACCG GGGACGAGTC CGACAACCTT CCCGGGGTCA AGGGCATCGG
 651 GGAGAAGACG GCGAGGAAGC TTCTGGAGGA GTGGGGGAGC CTGGAAGCCC
 701 TCCTCAAGAA CCTGGACCGG CTGAAGCCCG CCATCCGGGA GAAGATCCTG
 751 GCCCACATGG ACGATCTGAA GCTCTCCTGG GACCTGGCCA AGGTGCGCAC
 801 CGACCTGCCC CTGGAGGTGG ACTTCGCCAA AAGGCGGGAG CCCGACCGGG
 851 AGAGGCTTAG GGCCTTTCTG GAGAGGCTTG AGTTTGGCAG CCTCCTCCAC
 901 GAGTTCGGCC TTCTGGAAAG CCCCCCCGTT GGATACAGAA TAGTGAAAGA
 951 CCTGGTGGAA TTTGAAAAAC TCATAGAGAA ACTGAGAGAA TCCCCTTCGT
1001 TCGCCATAGA TCTTGAGACG TCTTCCCTCG ATCCTTTCGA CTGCGACATT
1051 GTCGGTATCT CTGTGTCTTT CAAACCAAAG GAAGCGTACT ACATACCACT
1101 CCATCATAGA AACGCCCAGA ACCTGGATGA AAAAGAAGTT CTGAAAAAGC
1151 TAAAAGAAAT CCTGGAGGAC CCCGGAGCAA AGATCGTTGG TCAGAATTTG
1201 AAATTCGATT ACAAGGTGTT GATGGTAAAG GGTGTTGAAC CTGTCCCTCC
1251 TCACTTCGAC ACGATGATAG CGGCTTACCT TCTTGAGCCG AACGAAAAGA
1301 AGTTCAATCT GGACGATCTC GCATTGAAAT TTCTTGGATA CAAAATGACC
1351 TCTTACCAGG AACTCATGTC CTTCTCTTCT CCGCTGTTTG GTTTCAGTTT
1401 TGCCGATGTT CCTGTAGAAA AAGCAGCGAA CTATTCCTGT GAAGATGCCG
1451 ACATCACCTA CAGACTCTAC AAGATCCTGA GCTTAAAACT CCACGAGGAG
1501 AGGCTCCTTT GGCTTTACCG GGAGGTGGAG AGGCCCCTTT CCGCTGTCCT
1551 GGCCCACATG GAGGCCACGG GGGTGCGCCT GGACGTGGCC TATCTCAGGG
1601 CCTTGTCCCT GGAGGTGGCC GAGGAGATCG CCCGCCTCGA GGCCGAGGTC
1651 TTCCGCCTGG CCGGCCACCC CTTCAACCTC AACTCCCGGG ACCAGCTGGA
1701 AAGGGTCCTC TTTGACGAGC TAGGGCTTCC CGCCATCGGC AAGACGGAGA
1751 AGACCGGCAA GCGCTCCACC AGCGCCGCCG TCCTGGAGGC CCTCCGCGAG
1801 GCCCACCCCA TCGTGGAGAA GATCCTGCAG TACCGGGAGC TCACCAAGCT
1851  GAAGAGCACC TACATTGACC CCTTGCCGGA CCTCATCCAC CCCAGGACGG
1901 GCCGCCTCCA CACCCGCTTC AACCAGACGG CCACGGCCAC GGGCAGGCTA
1951 AGTAGCTCCG ATCCCAACCT CCAGAACATC CCCGTCCGCA CCCCGCTTGG
2001 GCAGAGGATC CGCCGGGCCT TCATCGCCGA GGAGGGGTGG CTATTGGTGG
2051 CCCTGGACTA TAGCCAGATA GAGCTCAGGG TGCTGGCCCA CCTCTCCGGC
2101 GACGAGAACC TGATCCGGGT CTTCCAGGAG GGGCGGGACA TCCACACGGA
2151 GACCGCCAGC TGGATGTTCG GCGTCCCCCG GGAGGCCGTG GACCCCCTGA
2201 TGCGCCGGGC GGCCAAGACC ATCAACTTCG GGGTCCTCTA CGGCATGTCG
2251 GCCCACCGCC TCTCCCAGGA GCTAGCCATC CCTTACGAGG AGGCCCAGGC
2301 CTTCATTGAG CGCTACTTTC AGAGCTTCCC CAAGGTGCGG GCCTGGATTG
2351 AGAAGACCCT GGAGGAGGGC AGGAGGCGGG GGTACGTGGA GACCCTCTTC
2401 GGCCGCCGCC GCTACGTGCC AGACCTAGAG GCCCGGGTGA AGAGCGTGCG
2451 GGAGGCGGCC GAGCGCATGG CCTTCAACAT GCCCGTCCAG GGCACCGCCG
2501 CCGACCTCAT GAAGCTGGCT ATGGTGAAGC TCTTCCCCAG GCTGGAGGAA
```

Figure 3/2
SEQ ID No.: 3

```
2551 ATGGGGGCCA GGATGCTCCT TCAGGTCCAC GACGAGCTGG TCCTCGAGGC
2601 CCCAAAAGAG AGGGCGGAGG CCGTGGCCCG GCTGGCCAAG GAGGTCATGG
2651 AGGGGGTGTA TCCCCTGGCC GTGCCCCTGG AGGTGGAGGT GGGGATAGGG
2701 GAGGACTGGC TCTCCGCCAA GGAGTGA
```

SEQ ID No.: 9

amino acid sequence:

```
1    MRGSHHHHHH AADDDDKMRG MLPLFEPKGR VLLVDGHHLA YRTFHALKGL
51   TTSRGEPVQA VYGFAKSLLK ALKEDGDAVI VVFDAKAPSF RHEAYGGYKA
101  GRAPTPEDFP RQLALIKELV DLLGLARLEV PGYEADDVLA SLAKKAEKEG
151  YEVRILTADK DLYQLLSDRI HVLHPEGYLI TPAWLWEKYG LRPDQWADYR
201  ALTGDESDNL PGVKGIGEKT ARKLLEEWGS LEALLKNLDR LKPAIREKIL
251  AHMDDLKLSW DLAKVRTDLP LEVDFAKRRE PDRERLRAFL ERLEFGSLLH
301  EFGLLESPPV GYRIVKDLVE FEKLIEKLRE SPSFAIDLET SSLDPFDCDI
351  VGISVSFKPK EAYYIPLHHR NAQNLDEKEV LKKLKEILED PGAKIVGQNL
401  KFDYKVLMVK GVEPVPPHFD TMIAAYLLEP NEKKFNLDDL ALKFLGYKMT
451  SYQELMSFSS PLFGFSFADV PVEKAANYSC EDADITYRLY KILSLKLHEE
501  RLLWLYREVE RPLSAVLAHM EATGVRLDVA YLRALSLEVA EEIARLEAEV
551  FRLAGHPFNL NSRDQLERVL FDELGLPAIG KTEKTGKRST SAAVLEALRE
601  AHPIVEKILQ YRELTKLKST YIDPLPDLIH PRTGRLHTRF NQTATATGRL
651  SSSDPNLQNI PVRTPLGQRI RRAFIAEEGW LLVALDYSQI ELRVLAHLSG
701  DENLIRVFQE GRDIHTETAS WMFGVPREAV DPLMRRAAKT INFGVLYGMS
751  AHRLSQELAI PYEEAQAFIE RYFQSFPKVR AWIEKTLEEG RRRGYVETLF
801  GRRRYVPDLE ARVKSVREAA ERMAFNMPVQ GTAADLMKLA MVKLFPRLEE
851  MGARMLLQVH DELVLEAPKE RAEAVARLAK EVMEGVYPLA VPLEVEVGIG
901  EDWLSAKE
```

Figure 4/1
SEQ ID No.: 4

DNA sequence:

```
   1 ATGAGGGGCT CGCATCACCA TCACCATCAC GCTGCTGACG ATGACGATAA
  51 AATGAGGGGC ATGCTACCGC TATTTGAGCC CAAGGGCCGG GTCCTCCTGG
 101 TCGACGGCCA CCACCTGGCC TACCGCACCT TCCACGCCCT GAAGGGCCTC
 151 ACCACCAGCC GGGGGGAGCC GGTGCAGGCG GTCTACGGCT TCGCCAAGAG
 201 CCTCCTCAAG GCCCTCAAGG AGGACGGGGA CGCGGTGATC GTGGTCTTTG
 251 ACGCCAAGGC CCCCTCCTTC CGCCACGAGG CCTACGGGGG GTACAAGGCG
 301 GGCCGGGCCC CCACGCCGGA GGACTTTCCC CGGCAACTCG CCCTCATCAA
 351 GGAGCTGGTG GACCTCCTGG GGCTGGCGCG CCTCGAGGTC CCGGGCTACG
 401 AGGCGGACGA CGTCCTGGCC AGCCTGGCCA AGAAGGCGGA AAAGGAGGGC
 451 TACGAGGTCC GCATCCTCAC CGCCGACAAA GACCTTTACC AGCTCCTTTC
 501 CGACCGCATC CACGTCCTCC ACCCCGAGGG GTACCTCATC ACCCCGGCCT
 551 GGCTTTGGGA AAAGTACGGC CTGAGGCCCG ACCAGTGGGC CGACTACCGG
 601 GCCCTGACCG GGGACGAGTC CGACAACCTT CCCGGGGTCA AGGGCATCGG
 651 GGAGAAGACG GCGAGGAAGC TTCTGGAGGA GTGGGGGAGC CTGGAAGCCC
 701 TCCTCAAGAA CCTGGACCGG CTGAAGCCCG CCATCCGGGA GAAGATCCTG
 751 GCCCACATGG ACGATCTGAA GCTCTCCTGG GACCTGGCCA AGGTGCGCAC
 801 CGACCTGCCC CTGGAGGTGG ACTTCGCCAA AAGGCGGGAG CCCGACCGGG
 851 AGAGGCTTAG GGCCTTTCTG GAGAGGCTTG AGTTTGGCAG CCTCCTCCAC
 901 GAGTTCGGCC TTCTGGAAAG CCCCCCCGTT GGATACAGAA TAGTGAAAGA
 951 CCTGGTGGAA TTTGAAAAAC TCATAGAGAA ACTGAGAGAA TCCCCTTCGT
1001 TCGCCATAGA TCTTGAGACG TCTTCCCTCG ATCCTTTCGA CTGCGACATT
1051 GTCGGTATCT CTGTGTCTTT CAAACCAAAG GAAGCGTACT ACATACCACT
1101 CCATCATAGA AACGCCCAGA ACCTGGATGA AAAAGAAGTT CTGAAAAAGC
1151 TAAAAGAAAT CCTGGAGGAC CCCGGAGCAA AGATCGTTGG TCAGAATTTG
1201 AAATTCGATT ACAAGGTGTT GATGGTAAAG GGTGTTGAAC CTGTCCCTCC
1251 TCACTTCGAC ACGATGATAG CGGCTTACCT TCTTGAGCCG AACGAAAAGA
1301 AGTTCAATCT GGACGATCTC GCATTGAAAT TTCTTGGATA CAAAATGACC
1351 TCTTACCAGG AACTCATGTC CTTCTCTTCT CCGCTGTTTG GTTTCAGTTT
1401 TGCCGATGTT CCTGTAGAAA AAGCAGCGAA CTATTCCTGT GAAGATGCAG
1451 ACATCACCTA CAGACTCTAC AAGATCCTGA GCTTAAAACT CCACGAGGCA
1501 GATCTGGAGA ACGTGTTCTA CAAGATAGAA ATGCCTCTTG TGAGCGTGCT
1551 TGCACGGATG GAACTGAACG GTGTGCGCCT GGACGTGGCC TATCTCAGGG
1601 CCTTGTCCCT GGAGGTGGCC GAGGAGATCG CCCGCCTCGA GGCCGAGGTC
1651 TTCCGCCTGG CCGGCCACCC CTTCAACCTC AACTCCCGGG ACCAGCTGGA
1701 AAGGGTCCTC TTTGACGAGC TAGGGCTTCC CGCCATCGGC AAGACGGAGA
1751 AGACCGGCAA GCGCTCTACC AGCGCCGCCG TCCTGGAGGC CCTCCGCGAG
1801 GCCCACCCCA TCGTGGAGAA GATCCTGCAG TACCGGGAGC TCACCAAGCT
1851 GAAGAGCACC TACATTGACC CCTTGCCGGA CCTCATCCAC CCCAGGACGG
1901 GCCGCCTCCA CACCCGCTTC AACCAGACGG CCACGGCCAC GGGCAGGCTA
1951 AGTAGCTCCG ATCCCAACCT CCAGAACATC CCCGTCCGCA CCCCGCTTGG
2001 GCAGAGGATC CGCCGGGCCT TCATCGCCGA GGAGGGGTGG CTATTGGTGG
2051 CCCTGGACTA TAGCCAGATA GAGCTCAGGG TGCTGGCCCA CCTCTCCGGC
2101 GACGAGAACC TGATCCGGGT CTTCCAGGAG GGGCGGGACA TCCACACGGA
2151 GACCGCCAGC TGGATGTTCG GCGTCCCCCG GGAGGCCGTG GACCCCCTGA
2201 TGCGCCGGGC GGCCAAGACC ATCAACTTCG GGGTCCTCTA CGGCATGTCG
2251 GCCCACCGCC TCTCCCAGGA GCTAGCCATC CCTTACGAGG AGGCCCAGGC
2301 CTTCATTGAG CGCTACTTTC AGAGCTTCCC CAAGGTGCGG GCCTGGATTG
2351 AGAAGACCCT GGAGGAGGGC AGGAGGCGGG GGTACGTGGA GACCCTCTTC
2401 GGCCGCCGCC GCTACGTGCC AGACCTAGAG GCCCGGGTGA AGAGCGTGCG
2451 GGAGGCGGCC GAGCGCATGG CCTTCAACAT GCCCGTCCAG GGCACCGCCG
2501 CCGACCTCAT GAAGCTGGCT ATGGTGAAGC TCTTCCCCAG GCTGGAGGAA
```

Figure 4/2
SEQ ID No.: 4

```
2551  ATGGGGGCCA GGATGCTCCT TCAGGTCCAC GACGAGCTGG TCCTCGAGGC
2601  CCCAAAAGAG AGGGCGGAGG CCGTGGCCCG GCTGGCCAAG GAGGTCATGG
2651  AGGGGGTGTA TCCCCTGGCC GTGCCCCTGG AGGTGGAGGT GGGGATAGGG
2701  GAGGACTGGC TCTCCGCCAA GGAGTGA
```

SEQ ID NO.: 10

amino acid sequence:

```
  1   MRGSHHHHHH AADDDDKMRG MLPLFEPKGR VLLVDGHHLA YRTFHALKGL
 51   TTSRGEPVQA VYGFAKSLLK ALKEDGDAVI VVFDAKAPSF RHEAYGGYKA
101   GRAPTPEDFP RQLALIKELV DLLGLARLEV PGYEADDVLA SLAKKAEKEG
151   YEVRILTADK DLYQLLSDRI HVLHPEGYLI TPAWLWEKYG LRPDQWADYR
201   ALTGDESDNL PGVKGIGEKT ARKLLEEWGS LEALLKNLDR LKPAIREKIL
251   AHMDDLKLSW DLAKVRTDLP LEVDFAKRRE PDRERLRAFL ERLEFGSLLH
301   EFGLLESPPV GYRIVKDLVE FEKLIEKLRE SPSFAIDLET SSLDPFDCDI
351   VGISVSFKPK EAYYIPLHHR NAQNLDEKEV LKKLKEILED PGAKIVGQNL
401   KFDYKVLMVK GVEPVPPHFD TMIAAYLLEP NEKKFNLDDL ALKFLGYKMT
451   SYQELMSFSS PLFGFSFADV PVEKAANYSC EDADITYRLY KILSLKLHEA
501   DLENVFYKIE MPLVSVLARM ELNGVRLDVA YLRALSLEVA EEIARLEAEV
551   FRLAGHPFNL NSRDQLERVL FDELGLPAIG KTEKTGKRST SAAVLEALRE
601   AHPIVEKILQ YRELTKLKST YIDPLPDLIH PRTGRLHTRF NQTATATGRL
651   SSSDPNLQNI PVRTPLGQRI RRAFIAEEGW LLVALDYSQI ELRVLAHLSG
701   DENLIRVFQE GRDIHTETAS WMFGVPREAV DPLMRRAAKT INFGVLYGMS
751   AHRLSQELAI PYEEAQAFIE RYFQSFPKVR AWIEKTLEEG RRRGYVETLF
801   GRRRYVPDLE ARVKSVREAA ERMAFNMPVQ GTAADLMKLA MVKLFPRLEE
851   MGARMLLQVH DELVLEAPKE RAEAVARLAK EVMEGVYPLA VPLEVEVGIG
901   EDWLSAKE
```

Figure 5/1
SEQ ID No.: 5

DNA sequence:

```
   1 ATGAGGGGCT CGCATCACCA TCACCATCAC GCTGCTGACG ATGACGATAA
  51 AATGAGGGGC ATGCTACCGC TATTTGAGCC CAAGGGCCGG GTCCTCCTGG
 101 TCGACGGCCA CCACCTGGCC TACCGCACCT TCCACGCCCT GAAGGGCCTC
 151 ACCACCAGCC GGGGGGAGCC GGTGCAGGCG GTCTACGGCT TCGCCAAGAG
 201 CCTCCTCAAG GCCCTCAAGG AGGACGGGGA CGCGGTGATC GTGGTCTTTG
 251 ACGCCAAGGC CCCCTCCTTC CGCCACGAGG CCTACGGGGG GTACAAGGCG
 301 GGCCGGGCCC CCACGCCGGA GGACTTTCCC CGGCAACTCG CCCTCATCAA
 351 GGAGCTGGTG GACCTCCTGG GGCTGGCGCG CCTCGAGGTC CCGGGCTACG
 401 AGGCGGACGA CGTCCTGGCC AGCCTGGCCA AGAAGGCGGA AAAGGAGGGC
 451 TACGAGGTCC GCATCCTCAC CGCCGACAAA GACCTTTACC AGCTCCTTTC
 501 CGACCGCATC CACGTCCTCC ACCCCGAGGG GTACCTCATC ACCCCGGCCT
 551 GGCTTTGGGA AAAGTACGGC CTGAGGCCCG ACCAGTGGGC CGACTACCGG
 601 GCCCTGACCG GGGACGAGTC CGACAACCTT CCCGGGGTCA AGGGCATCGG
 651 GGAGAAGACG GCGAGGAAGC TTCTGGAGGA GTGGGGGAGC CTGGAAGCCC
 701 TCCTCAAGAA CCTGGACCGG CTGAAGCCCG CCATCCGGGA GAAGATCCTG
 751 GCCCACATGG ACGATCTGAA GCTCTCCTGG GACCTGGCCA AGGTGCGCAC
 801 CGACCTGCCC CTGGAGGTGG ACTTCGCCAA AAGGCGGGAG CCCGACCGGG
 851 AGAGGCTTAG GGCCTTTCTG GAGAGGCTTG AGTTTGGCAG CCTCCTCCAC
 901 GAGTTCGGCC TTCTGGAAAG CCCCCATCCA GCAGTTGTGG ACATCTTCGA
 951 ATACGATATT CCATTTGCAA AGAGATACCT CATCGACAAA GGCCTAATAC
1001 CAATGGAGGG GGAAGAAGAG CTAAAGATTC TTGCCTTCGA TATAGAAACC
1051 CTCTATCACG AAGGAGAAGA GTTTGGAAAA GGCCCAATTA TAATGATTAG
1101 TTATGCAGAT GAAAATGAAG CAAAGGTGAT TACTTGGAAA AACATAGATC
1151 TTCCATACGT TGAGGTTGTA TCAAGCGAGA GAGAGATGAT AAAGAGATTT
1201 CTCAGGATTA TCAGGGAGAA GGATCCTGAC ATTATAGTTA CTTATAATGG
1251 AGACTCATTC GACTTCCCAT ATTTAGCGAA AAGGGCAGAA AAACTTGGGA
1301 TTAAATTAAC CATTGGAAGA GATGGAAGCG AGCCCAAGAT GCAGAGAATA
1351 GGCGATATGA CGGCTGTAGA AGTCAAGGGA AGAATACATT TCGACTTGTA
1401 TCATGTAATA ACAAGGACAA TAAATCTCCC AACATACACA CTAGAGGCTG
1451 TATATGAAGC AATTTTTGGA AAGCCAAAGG AGAAGGTATA CGCCGACGAG
1501 ATAGCAAAAG CCTGGGAAAG TGGAGAGAAC CTTGAGAGAG TTGCCAAATA
1551 CTCGATGGAA GATGCAAAGG CAACTTATGA ACTCGGGAAA GAATTCCTTC
1601 CAATGGAAAT TCAGCTTTCA GAGAGGCTCC TTTGGCTTTA CCGGGAGGTG
1651 GAGAGGCCCC TTTCCGCTGT CCTGGCCCAC ATGGAGGCCA CGGGGGTGCG
1701 CCTGGACGTG GCCTATCTCA GGGCCTTGTC CCTGGAGGTG GCCGAGGAGA
1751 TCGCCCGCCT CGAGGCCGAG GTCTTCCGCC TGGCCGGCCA CCCCTTCAAC
1801 CTCAACTCCC GGGACCAGCT GGAAAGGGTC CTCTTTGACG AGCTAGGGCT
1851 TCCCGCCATC GGCAAGACGG AGAAGACCGG CAAGCGCTCC ACCAGCGCCG
1901 CCGTCCTGGA GGCCCTCCGC GAGGCCCACC CCATCGTGGA GAAGATCCTG
1951 CAGTACCGGG AGCTCACCAA GCTGAAGAGC ACCTACATTG ACCCCTTGCC
2001 GGACCTCATC CACCCCAGGA CGGGCCGCCT CCACACCCGC TTCAACCAGA
2051 CGGCCACGGC CACGGGCAGG CTAAGTAGCT CCGATCCCAA CCTCCAGAAC
2101 ATCCCCGTCC GCACCCCGCT TGGGCAGAGG ATCCGCCGGG CCTTCATCGC
2151 CGAGGAGGGG TGGCTATTGG TGGCCCTGGA CTATAGCCAG ATAGAGCTCA
2201 GGGTGCTGGC CCACCTCTCC GGCGACGAGA ACCTGATCCG GGTCTTCCAG
2251 GAGGGGCGGG ACATCCACAC GGAGACCGCC AGCTGGATGT TCGGCGTCCC
2301 CCGGGAGGCC GTGGACCCCC TGATGCGCCG GGCGGCCAAG ACCATCAACT
2351 TCGGGGTCCT CTACGGCATG TCGGCCCACC GCCTCTCCCA GGAGCTAGCC
2401 ATCCCTTACG AGGAGGCCCA GGCCTTCATT GAGCGCTACT TTCAGAGCTT
2451 CCCCAAGGTG CGGGCCTGGA TTGAGAAGAC CCTGGAGGAG GGCAGGAGGC
2501 GGGGGTACGT GGAGACCCTC TTCGGCCGCC GCCGCTACGT GCCAGACCTA
```

Figure 5/2
SEQ ID No.: 5

```
2551  GAGGCCCGGG TGAAGAGCGT GCGGGAGGCG GCCGAGCGCA TGGCCTTCAA
2601  CATGCCCGTC CAGGGCACCG CCGCCGACCT CATGAAGCTG GCTATGGTGA
2651  AGCTCTTCCC CAGGCTGGAG GAAATGGGGG CCAGGATGCT CCTTCAGGTC
2701  CACGACGAGC TGGTCCTCGA GGCCCCAAAA GAGAGGGCGG AGGCCGTGGC
2751  CCGGCTGGCC AAGGAGGTCA TGGAGGGGGT GTATCCCCTG GCCGTGCCCC
2801  TGGAGGTGGA GGTGGGGATA GGGGAGGACT GGCTCTCCGC CAAGGAGTGA
```

SEQ ID No.: 11

amino acid sequence:

```
  1   MRGSHHHHHH AADDDDKMRG MLPLFEPKGR VLLVDGHHLA YRTFHALKGL
 51   TTSRGEPVQA VYGFAKSLLK ALKEDGDAVI VVFDAKAPSF RHEAYGGYKA
101   GRAPTPEDFP RQLALIKELV DLLGLARLEV PGYEADDVLA SLAKKAEKEG
151   YEVRILTADK DLYQLLSDRI HVLHPEGYLI TPAWLWEKYG LRPDQWADYR
201   ALTGDESDNL PGVKGIGEKT ARKLLEEWGS LEALLKNLDR LKPAIREKIL
251   AHMDDLKLSW DLAKVRTDLP LEVDFAKRRE PDRERLRAFL ERLEFGSLLH
301   EFGLLESPHP AVVDIFEYDI PFAKRYLIDK GLIPMEGEEE LKILAFDIET
351   LYHEGEEFGK GPIIMISYAD ENEAKVITWK NIDLPYVEVV SSEREMIKRF
401   LRIIREKDPD IIVTYNGDSF DFPYLAKRAE KLGIKLTIGR DGSEPKMQRI
451   GDMTAVEVKG RIHFDLYHVI TRTINLPTYT LEAVYEAIFG KPKEKVYADE
501   IAKAWESGEN LERVAKYSME DAKATYELGK EFLPMEIQLS ERLLWLYREV
551   ERPLSAVLAH MEATGVRLDV AYLRALSLEV AEEIARLEAE VFRLAGHPFN
601   LNSRDQLERV LFDELGLPAI GKTEKTGKRS TSAAVLEALR EAHPIVEKIL
651   QYRELTKLKS TYIDPLPDLI HPRTGRLHTR FNQTATATGR LSSSDPNLQN
701   IPVRTPLGQR IRRAFIAEEG WLLVALDYSQ IELRVLAHLS GDENLIRVFQ
751   EGRDIHTETA SWMFGVPREA VDPLMRRAAK TINFGVLYGM SAHRLSQELA
801   IPYEEAQAFI ERYFQSFPKV RAWIEKTLEE GRRRGYVETL FGRRRYVPDL
851   EARVKSVREA AERMAFNMPV QGTAADLMKL AMVKLFPRLE EMGARMLLQV
901   HDELVLEAPK ERAEAVARLA KEVMEGVYPL AVPLEVEVGI GEDWLSAKE*
```

Figure 6/1
SEQ ID No.: 6

DNA sequence:

```
   1  ATGAGGGGCT CGCATCACCA TCACCATCAC GCTGCTGACG ATGACGATAA
  51  AATGAGGGGC ATGCTACCGC TATTTGAGCC CAAGGGCCGG GTCCTCCTGG
 101  TCGACGGCCA CCACCTGGCC TACCGCACCT TCCACGCCCT GAAGGGCCTC
 151  ACCACCAGCC GGGGGGAGCC GGTGCAGGCG GTCTACGGCT TCGCCAAGAG
 201  CCTCCTCAAG GCCCTCAAGG AGGACGGGGA CGCGGTGATC GTGGTCTTTG
 251  ACGCCAAGGC CCCCTCCTTC CGCCACGAGG CCTACGGGGG GTACAAGGCG
 301  GGCCGGGCCC CCACGCCGGA GGACTTTCCC CGGCAACTCG CCCTCATCAA
 351  GGAGCTGGTG GACCTCCTGG GGCTGGCGCG CCTCGAGGTC CCGGGCTACG
 401  AGGCGGACGA CGTCCTGGCC AGCCTGGCCA AGAAGGCGGA AAAGGAGGGC
 451  TACGAGGTCC GCATCCTCAC CGCCGACAAA GACCTTTACC AGCTCCTTTC
 501  CGACCGCATC CACGTCCTCC ACCCCGAGGG GTACCTCATC ACCCCGGCCT
 551  GGCTTTGGGA AAAGTACGGC CTGAGGCCCG ACCAGTGGGC CGACTACCGG
 601  GCCCTGACCG GGGACGAGTC CGACAACCTT CCCGGGGTCA AGGGCATCGG
 651  GGAGAAGACG GCGAGGAAGC TTCTGGAGGA GTGGGGGAGC CTGGAAGCCC
 701  TCCTCAAGAA CCTGGACCGG CTGAAGCCCG CCATCCGGGA GAAGATCCTG
 751  GCCCACATGG ACGATCTGAA GCTCTCCTGG GACCTGGCCA AGGTGCGCAC
 801  CGACCTGCCC CTGGAGGTGG ACTTCGCCAA AAGGCGGGAG CCCGACCGGG
 851  AGAGGCTTAG GGCCTTTCTG GAGAGGCTTG AGTTTGGCAG CCTCCTCCAC
 901  GAGTTCGGCC TTCTGGAAAG CCCCGTTAGA GAACATCCAG CAGTTGTGGA
 951  CATCTTCGAA TACGATATTC CATTTGCAAA GAGATACCTC ATCGACAAAG
1001  GCCTAATACC AATGGAGGGG GAAGAAGAGC TAAAGATTCT TGCCTTCGAT
1051  ATAGAAACCC TCTATCACGA AGGAGAAGAG TTTGGAAAAG GCCCAATTAT
1101  AATGATTAGT TATGCAGATG AAAATGAAGC AAAGGTGATT ACTTGGAAAA
1151  ACATAGATCT TCCATACGTT GAGGTTGTAT CAAGCGAGAG AGAGATGATA
1201  AAGAGATTTC TCAGGATTAT CAGGGAGAAG GATCCTGACA TTATAGTTAC
1251  TTATAATGGA GACTCATTCG ACTTCCCATA TTTAGCGAAA AGGGCAGAAA
1301  AACTTGGGAT TAAATTAACC ATTGGAAGAG ATGGAAGCGA GCCCAAGATG
1351  CAGAGAATAG GCGATATGAC GGCTGTAGAA GTCAAGGGAA GAATACATTT
1401  CGACTTGTAT CATGTAATAA CAAGGACAAT AAATCTCCCA ACATACACAC
1451  TAGAGGCTGT ATATGAAGCA ATTTTTGGAA AGCCAAAGGA GAAGGTATAC
1501  GCCGACGAGA TAGCAAAAGC CTGGGAAAGT GGAGAGAACC TTGAGAGAGT
1551  TGCCAAATAC TCGATGGAAG ATGCAAAGGC AACTTATGAA CTCGGGAAAG
1601  AATTCCTTCC AATGGAAATT CAGCTTTCAA GATTAGTTGG ACAACCTTTA
1651  TGGGATGTTT CAAGGTCAAG CACAGGGAAC CTTGTAGAGT GGTTCTTACT
1701  TAGGAAAGCC TACGAAAGAA ACGAAGTAGC TCCAAACAAG CCAAGTGAAG
1751  AGGAGTATCA AAGAAGGCTC AGGGAGAGCT ACACAGGTGG ATTCGTGCGC
1801  CTGGACGTGG CCTATCTCAG GGCCTTGTCC CTGGAGGTGG CCGAGGAGAT
1851  CGCCCGCCTC GAGGCCGAGG TCTTCCGCCT GGCCGGCCAC CCCTTCAACC
1901  TCAACTCCCG GGACCAGCTG GAAAGGGTCC TCTTTGACGA GCTAGGGCTT
1951  CCCGCCATCG GCAAGACGGA GAAGACCGGC AAGCGCTCCA CCAGCGCCGC
2001  CGTCCTGGAG GCCCTCCGCG AGGCCCACCC CATCGTGGAG AAGATCCTGC
2051  AGTACCGGGA GCTCACCAAG CTGAAGAGCA CCTACATTGA CCCCTTGCCG
2101  GACCTCATCC ACCCCAGGAC GGGCCGCCTC CACACCCGCT TCAACCAGAC
2151  GGCCACGGCC ACGGGCAGGC TAAGTAGCTC CGATCCCAAC CTCCAGAACA
2201  TCCCCGTCCG CACCCCGCTT GGGCAGAGGA TCCGCCGGGC CTTCATCGCC
2251  GAGGAGGGGT GGCTATTGGT GGCCCTGGAC TATAGCCAGA TAGAGCTCAG
2301  GGTGCTGGCC CACCTCTCCG GCGACGAGAA CCTGATCCGG GTCTTCCAGG
2351  AGGGGCGGGA CATCCACACG GAGACCGCCA GCTGGATGTT CGGCGTCCCC
2401  CGGGAGGCCG TGGACCCCCT GATGCGCCGG GCGGCCAAGA CCATCAACTT
2451  CGGGGTCCTC TACGGCATGT CGGCCCACCG CCTCTCCCAG GAGCTAGCCA
2501  TCCCTTACGA GGAGGCCCAG GCCTTCATTG AGCGCTACTT TCAGAGCTTC
```

Figure 6/2
SEQ ID No.: 6

```
2551  CCCAAGGTGC GGGCCTGGAT TGAGAAGACC CTGGAGGAGG GCAGGAGGCG
2601  GGGGTACGTG GAGACCCTCT TCGGCCGCCG CCGCTACGTG CCAGACCTAG
2651  AGGCCCGGGT GAAGAGCGTG CGGGAGGCGG CCGAGCGCAT GGCCTTCAAC
2701  ATGCCCGTCC AGGGCACCGC CGCCGACCTC ATGAAGCTGG CTATGGTGAA
2751  GCTCTTCCCC AGGCTGGAGG AAATGGGGGC CAGGATGCTC CTTCAGGTCC
2801  ACGACGAGCT GGTCCTCGAG GCCCCAAAAG AGAGGGCGGA GGCCGTGGCC
2851  CGGCTGGCCA AGGAGGTCAT GGAGGGGGTG TATCCCCTGG CCGTGCCCCT
2901  GGAGGTGGAG GTGGGGATAG GGGAGGACTG GCTCTCCGCC AAGGAGTGA
```

SEQ ID No.: 12

amino acid sequence:

```
  1   MRGSHHHHHH AADDDDKMRG MLPLFEPKGR VLLVDGHHLA YRTFHALKGL
 51   TTSRGEPVQA VYGFAKSLLK ALKEDGDAVI VVFDAKAPSF RHEAYGGYKA
101   GRAPTPEDFP RQLALIKELV DLLGLARLEV PGYEADDVLA SLAKKAEKEG
151   YEVRILTADK DLYQLLSDRI HVLHPEGYLI TPAWLWEKYG LRPDQWADYR
201   ALTGDESDNL PGVKGIGEKT ARKLLEEWGS LEALLKNLDR LKPAIREKIL
251   AHMDDLKLSW DLAKVRTDLP LEVDFAKRRE PDRERLRAFL ERLEFGSLLH
301   EFGLLESPVR EHPAVVDIFE YDIPFAKRYL IDKGLIPMEG EEELKILAFD
351   IETLYHEGEE FGKGPIIMIS YADENEAKVI TWKNIDLPYV EVVSSEREMI
401   KRFLRIIREK DPDIIVTYNG DSFDFPYLAK RAEKLGIKLT IGRDGSEPKM
451   QRIGDMTAVE VKGRIHFDLY HVITRTINLP TYTLEAVYEA IFGKPKEKVY
501   ADEIAKAWES GENLERVAKY SMEDAKATYE LGKEFLPMEI QLSRLVGQPL
551   WDVSRSSTGN LVEWFLLRKA YERNEVAPNK PSEEEYQRRL RESYTGGFVR
601   LDVAYLRALS LEVAEEIARL EAEVFRLAGH PFNLNSRDQL ERVLFDELGL
651   PAIGKTEKTG KRSTSAAVLE ALREAHPIVE KILQYRELTK LKSTYIDPLP
701   DLIHPRTGRL HTRFNQTATA TGRLSSSDPN LQNIPVRTPL GQRIRRAFIA
751   EEGWLLVALD YSQIELRVLA HLSGDENLIR VFQEGRDIHT ETASWMFGVP
801   REAVDPLMRR AAKTINFGVL YGMSAHRLSQ ELAIPYEEAQ AFIERYFQSF
851   PKVRAWIEKT LEEGRRRGYV ETLFGRRRYV PDLEARVKSV REAAERMAFN
901   MPVQGTAADL MKLAMVKLFP RLEEMGARML LQVHDELVLE APKERAEAVA
951   RLAKEVMEGV YPLAVPLEVE VGIGEDWLSA KE*
```

Figure 7

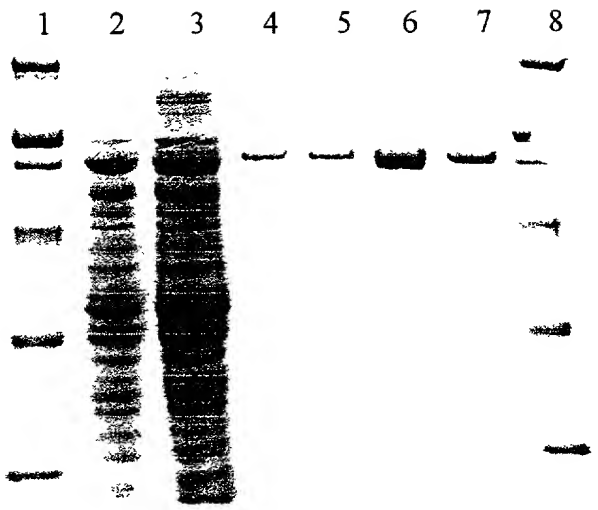


Figure 8

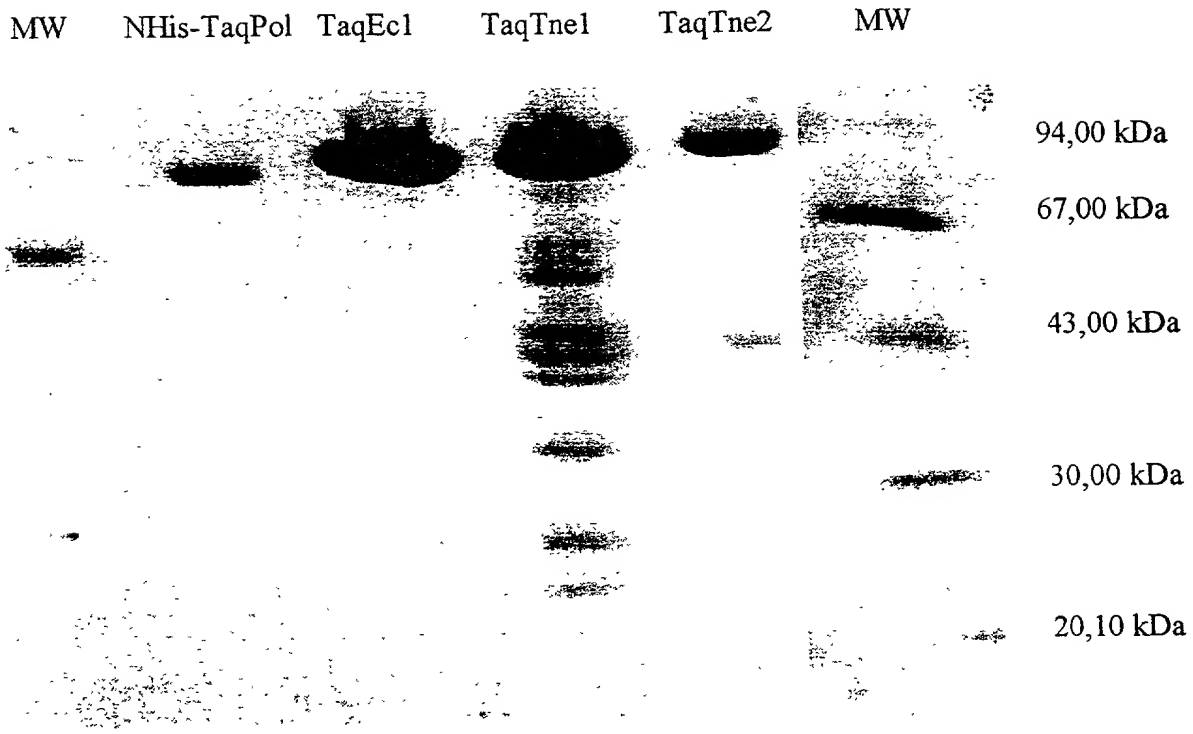

MW
NHis-TaqPol
TaqEc1
TaqTne1
TaqTne2
MW
94,00 kDa
67,00 kDa
43,00 kDa
30,00 kDa
20,10 kDa

Figure 9

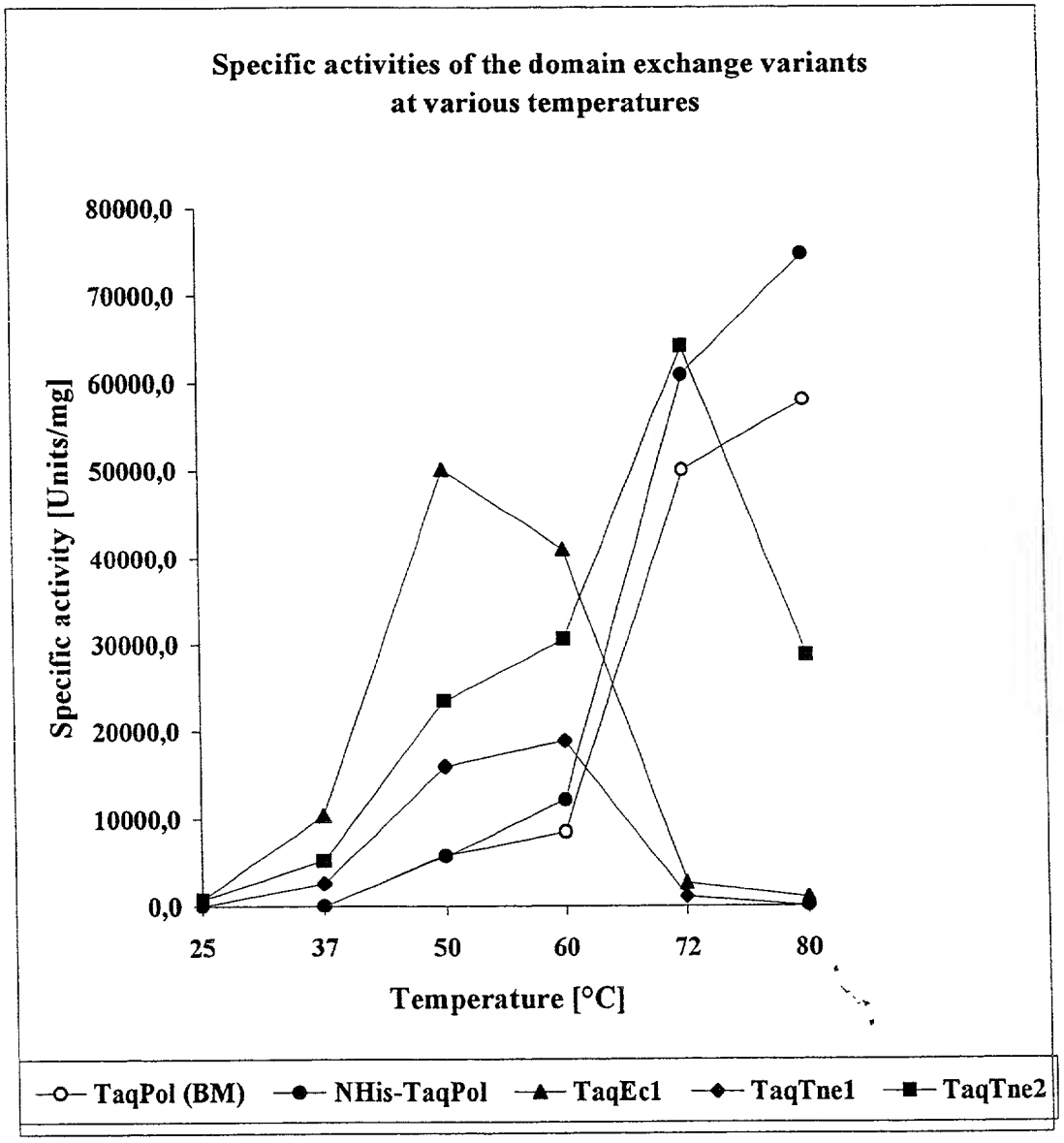

Specific activities of the domain exchange variants at various temperatures
Specific activity [Units/mg]
80000,0
70000,0
60000,0
50000,0
40000,0
30000,0
20000,0
10000,0
0,0
25
37
50
60
72
80
Temperature [°C]
TaqPol (BM)
NHis-TaqPol
TaqEc1
TaqTne1
TaqTne2

Figure 10

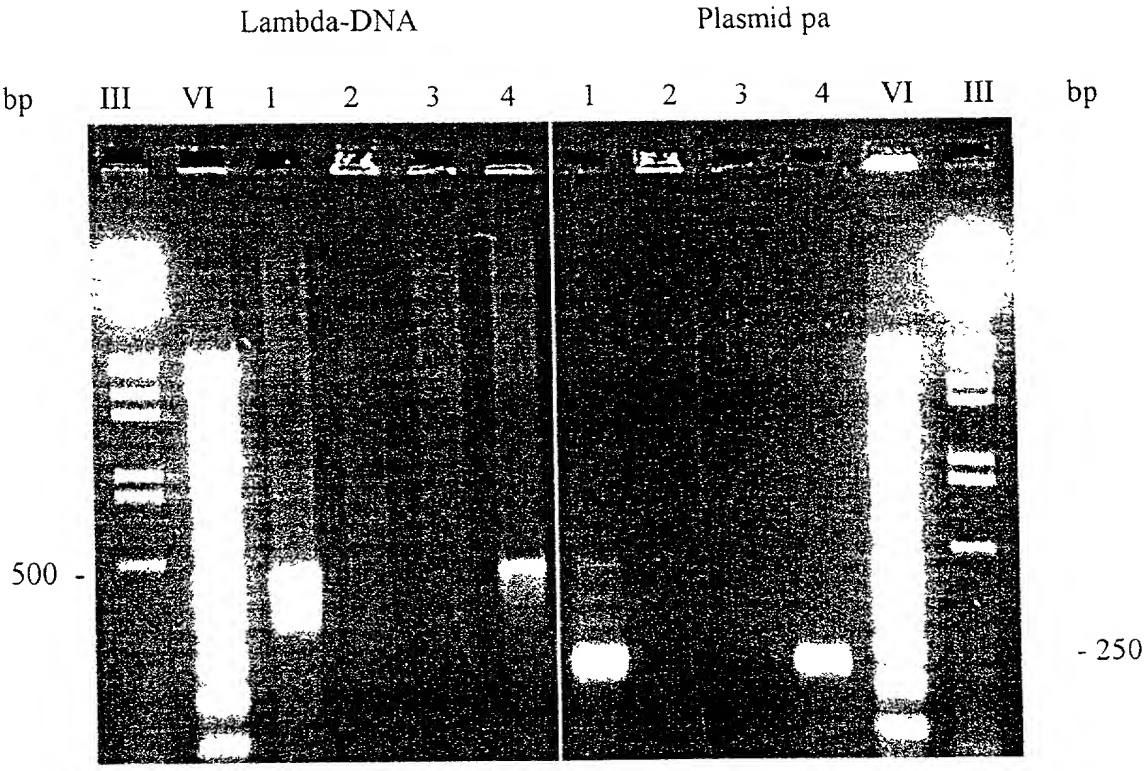


Figure 11

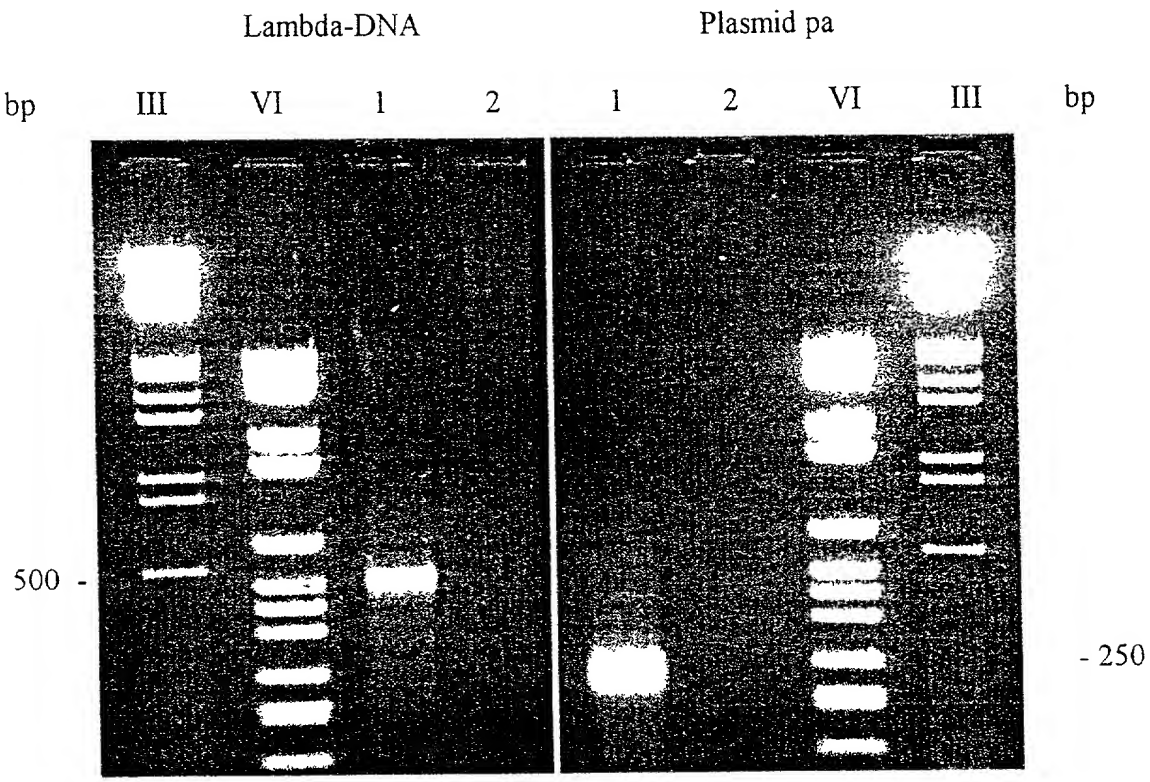


Figure 12

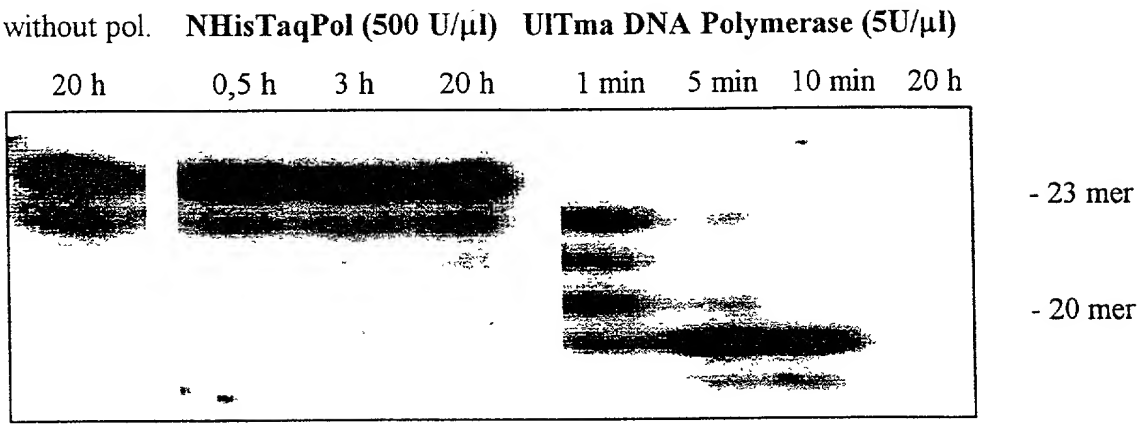

without pol.
NHisTaqPol (500 U/µl)
UlTma DNA Polymerase (5U/µl)
20 h
0,5 h
3 h
20 h
1 min
5 min
10 min
20 h
- 23 mer
- 20 mer

Figure 13

without pol. **TaqEc1 (500 U/µl)**

600 15 30 45 60 90 180 600 min

ohne Pol **TaqEc1 (500 U/µl)**

600 15 30 45 60 90 180 600 min

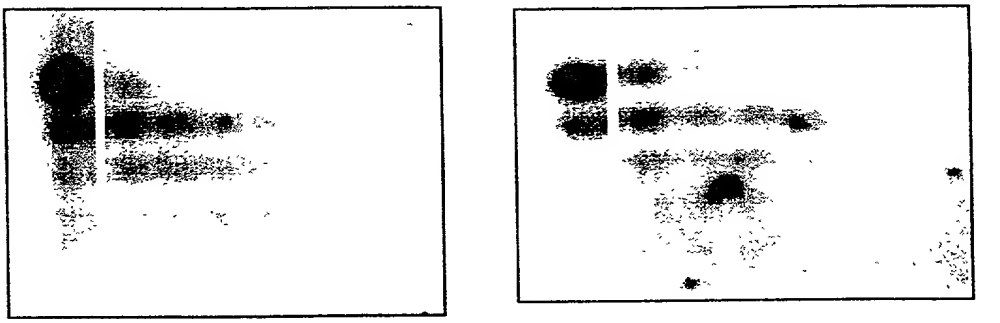


Figure 14

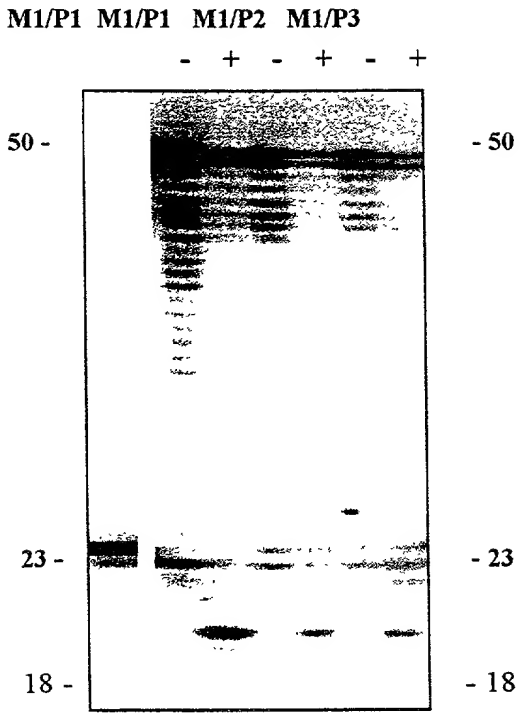


Figure 15

Degradation of primers at the 3'-end (3'-5' exonuclease assay)
and
Correction of 3'-mismatched Primers and their extension (3'-mismatch primer correction assay)

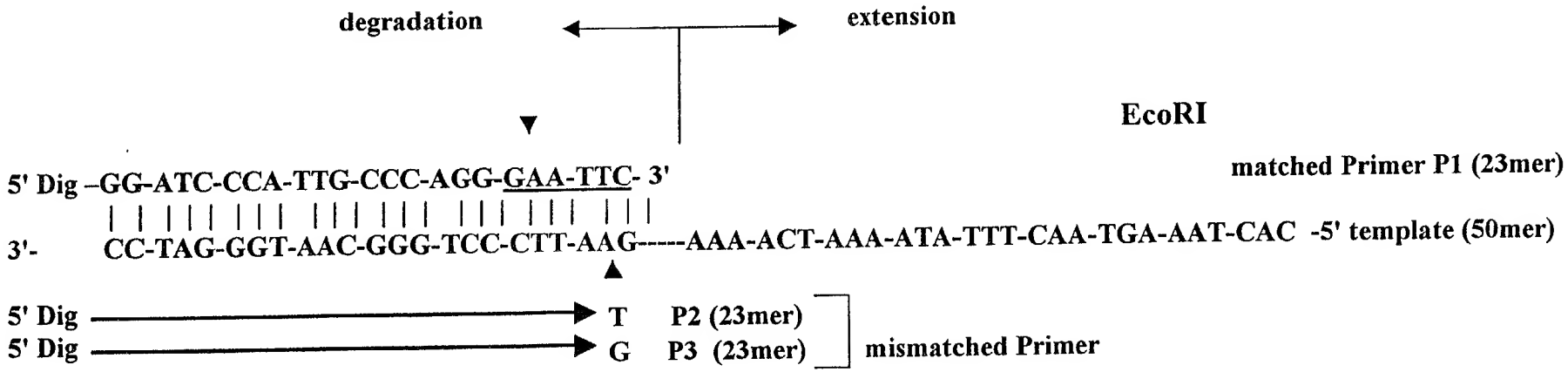


Figure 16

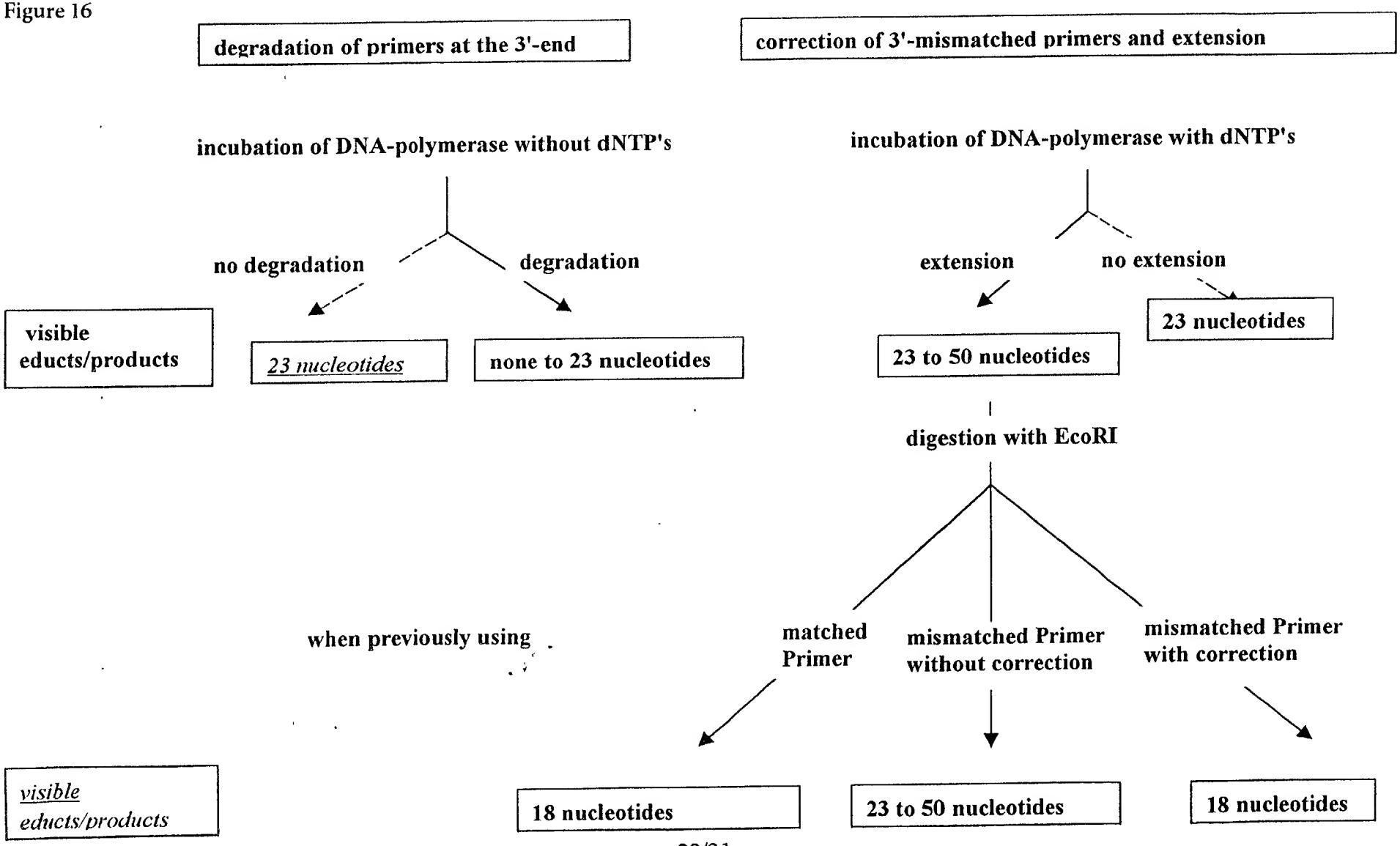


Fig. 17/1

SEQ ID No. 43
SEQ ID No. 44
SEQ ID No. 45
SEQ ID No. 46

```
chimera___   -----MARLFLFDGTALAYRAYYALDRSLSTSTGIPTNATYGVARMLVRFIKDHIIVGKD
tne.rse___   -----MARLFLFDGTALAYRAYYALDRSLSTSTGIPTNATYGVARMLVRFIKDHIIVGKD
ath.rse___   ------MKLVIFDGNSILYRAFFALP-ELTTSNNIPTNAIYGFVNVILKYLEQ---EKPD
DPO1_ECOLI   MVQIPQNPLILVDGSSYLYRAYHAFP-PLTNSAGEPTGAMYGVLNMLRSLIMQ---YKPT
                     * . ** .  ***. *    *..* . **.* **   ..   .

chimera___   YVAVAFDKKAATFRHKLLETYKAQRPKTPDLLIQQLPYIKKLVEALGMKVLEVEGYEADD
tne.rse___   YVAVAFDKKAATFRHKLLETYKAQRPKTPDLLIQQLPYIKKLVEALGMKVLEVEGYEADD
ath.rse___   YVAVAFDKRGREARKSEYEEYKANRKPMPDNLQVQIPYVREILYAFNIPIIEFEGYEADD
DPO1_ECOLI   HAAVVFDAKGKTFRDELFEHYKSHRPPMPDDLRAQIEPLHAMVKAMGLPLLAVSGVEADD
               ** ** .    *    * **. *   ** *  *.  .. .. * .. ..   * ****

chimera___   IIATLAVKGLPLFDEIFIVTGDKDMLQLVNEKIKVWRIVKGISD--LELYDAQKVKEKYG
chimera___   IIATLAVKGLPLFDEIFIVTGDKDMLQLVNEKIKVWRIVKGISD--LELYDAQKVKEKYG
ath.rse___   VIGSLVNQFKNTGLDIVIITGDRDTLQLLDKNVVVKIVSTKFDKTVEDLYTVENVKEKYG
DPO1_ECOLI   VIGTLAREAEKAGRPVLISTGDKDMAQLVTPNITLINTMTNTILG--PE----EVVNKYG
             .* .*          . * ***.*  **.   . .                    *  ***

chimera___   VEPQQIPDLLALTGDEIDNIPGVTGIGEKTAVQLLEKYKDLEDILNHVRELP-------Q
tne.rse___   VEPQQIPDLLALTGDEIDNIPGVTGIGEKTAVQLLEKYKDLEDILNHVRELP-------Q
ath.rse___   VWANQVPDYKALVGDQSDNIPGVKGIGEKSAQKLLEEYSSLEEIYQNLDKIK-------S
DPO1_ECOLI   VPPELIIDFLALMGDSSDNIPGVPGVGEKTAQALLQGLGGLDTLYAEPEKIAGLSFRGAK
             *    . *  ** **  ****** *.***.*  **.    *. .      .

chimera___   KVRKALLRDRENAILSKKLAILETNVPIEINWEELRYQGYDREKLLPLLKELEFASIMKE
tne.rse___   KVRKALLRDRENAILSKKLAILETNVPIEINWEELRYQGYDREKLLPLLKELEFASIMKE
ath.rse___   SIREKLEAGKDMAFLSKRLATIVCDLPLNVKLEDLRTKEWNKERLYEILVQLEFKSIIKR
DPO1_ECOLI   TMAAKLEQNKEVAYLSYQLATIKTDVELELTCEQLEVQQPAAEELLGLFKKYEFKRWTAD
              .   *   .. * ** .** . . . . .  * *  .    * *  .    **

chimera___   LQLYEESEPVGYRIVK-------------------------------DLVEFEKLIEKLRESP
tne.rse___   LQLYEESEPVGYRIVK-------------------------------DLVEFEKLIEKLRESP
ath.rse___   LGLS-----------------------------------------EVVQFEFVQQRTDIPD
DPO1-ECOLI   VEAGKWLQAKGAKPAAKPQETSVADEAPEVTATVISYDNYVTILDEETLKAWIAKLEKAP
             .                                                .        .

chimera___   SFAIDLETSSLDPFDCDIVGISVSFKPKEAYYIPLHHRNAQNLDEKE---VLKKLKEILE
tne.rse___   SFAIDLETSSLDPFDCDIVGISVSFKPKEAYYIPLHHRNAQNLDEKE---VLKKLKEILE
ath.rse___   VEQKELESISQIRSKE--IPLMFVQGEK-CFYLYDQESNTVFITSN------KLLIEEIL
DPO1_ECOLI   VFAFDTETDSLDNISANLVGLSFAIEPGVAAYIPVAHDYLDAPDQISRERALELLKPLLE
                 . *. *       . .       . *.                    *   .
```

Fig. 17/2

```
chimera___   DPGAKIVGQNLKFDYKVLMVKGVEPVPPHFDTMIAAYLLEPNEKKFNLDDLALKFLGYKM
tne.rse___   DPGAKIVGQNLKFDYKVLMVKGVEPVPPHFDTMIAAYLLEPNEKKFNLDDLALKFLGYKM
ath.rse___   KSDTVKIMYDLKNIFHQLNLEDTNNIKNCEDVMIASYVLDSTRSSYELETLFVSYLNTDI
DPO1_ECOLI   DEKALKVGQNLKYDRGILANYGIELRGIAFDTMLESYILNSVAGRHDMDSLAERWLKHKT
                .  .   **     *              * *. .*.*         .. *    *

chimera___   TSYQELMSFSSPLFGFSFADVPVEKAANYSCEDADITYRLYKILSLKLHEAD-LENVFYK
tne.rse___   TSYQELMSFSSPLFGFSFADVPVEKAANYSCEDADITYRLYKILSLKLHEAD-LENVFYK
ath.rse___   EAVKKDKKIVS----------------------VVLLKRLWDELLRLIDLNS-CQFLYEN
DPO1_ECOLI   ITFEEIAGKGKNQ--LTFNQIALEEAGRYAAEDADVTLQLHLKMWPDLQKHKGPLNVFEN
              .                                      .  .*   .   .        ..

chimera___   IEMPLVSVLARMELNGVKVDRDALIQYTKEIENKILKLETQIYQIAGEWFNINSPKQLSY
tne.rse___   IEMPLVSVLARMELNGVYVDTEFLKKLSEEYGKKLEELAEEIYRIAGEPFNINSPKQVSR
ath.rse___   IERPLIPVLYEMEKTGFKVDRDALIQYTKEIENKILKLETQIYQIAGEWFNINSPKQLSY
DPO1-ECOLI   IEMPLVPVLSRIERNGVKIDPKVLHNHSEELTLRLAELEKKAHEIAGEEFNLSSTKQLQT
             ** **. **  .* .*  .*   *   . *   ..  *      **** **. * **.

chimera___   ILFEKLKLPVIKKTKTG--YSTDAEVLEELFDKHEIVPLILDYRMYTKILTTYCQGLLQA
tne.rse___   ILFEKLGIKPRGKTTKTGDYSTRIEVLEELAGEHEIIPLILEYRKIQKLKSTYIDALPKM
ath.rse___   ILFEKLKLPVIKKTKTG--YSTDAEVLEELFDKHEIVPLILDYRMYTKILTTYCQGLLQA
DPO1_ECOLI   ILFEKQGIKPLKKTPGG-APSTSEEVLEELALDYPLPKVILEYRGLAKLKSTYTDKLPLM
             *****  .    **     **  ******     .  .**.**   *. .**   *

chimera___   INPSSGRVHTTFIQTGTATGRLASSDPNLQNIPVKYDEGKLIRKVFVPEG-GHVLIDADY
tne.rse___   VNPKTGRIHASFNQTGTATGRLSSSDPNLQNLPTKSEEGKEIRKAIVPQDPNWWIVSADY
ath.rse___   INPSSGRVHTTFIQTGTATGRLASSDPNLQNIPVKYDEGKLIRKVFVPEG-GHVLIDADY
DPO1-ECOLI   INPKTGRVHTSYHQAVTATGRLSSTDPNLQNIPVRNEEGRRIRQAFIAPE-DYVIVSADY
             .** .**.*... *. ******.*.******.* . .**. **.  .        .. ***

chimera___   SQIELRILAHISEDERLISAFKNNVDIHSQTAAEVFGVDIADVTPEMRSQAKAVNFGIVY
tne.rse___   SQIELRILAHLSGDENLLRAFEEGIDVHTLTASRIFNVKPEEVTEEMRRAGKMVNFSIIY
ath.rse___   SQIELRILAHISEDERLISAFKNNVDIHSQTAAEVFGVDIADVTPEMRSQAKAVNFGIVY
DPO1_ECOLI   SQIELRIMAHLSRDKGLLTAFAEGKDIHRATAAEVFGLPLETVTSEQRRSAKAINFGLIY
             *******.**.* *  *. **  . *.*  **. .*..     ** * *   * .** ..*
                                                        .

chimera___   GISDYGLARDIKISRKEAAEFINKYFERYPKVKEYLDNTVKFARDNGFVLTLFNRKRYIK
tne.rse___   GVTPYGLSVRLGVPVKEAEKMIVNYFVLYPKVRDYIQRVVSEAKEKGYVRTLFGRKRDIP
ath.rse___   GISDYGLARDIKISRKEAAEFINKYFERYPKVKEYLDNTVKFARDNGFVLTLFNRKRYIK
DPO1_ECOLI   GMSAFGLARQLNIPRKEAQKYMDLYFERYPGVLEYMERTRAQAKEQGYVETLDGRRLYLP
             *.. .**.  . .  ***   .  **  ** * .*.      *.. *.* ** .*.  .

chimera___   DIKSTNRNLRGYAERIAMNSPIQGSAADIMKLAMIKVYQKLKENNLKSKIILQVHDELLI
tne.rse___   QLMARDRNTQAEGERIAINTPIQGTAADIIKLAMIEIDRELKERKMRSKMIIQVHDELVF
ath.rse___   DIKSTNRNLRGYAERIAMNSPIQGSAADIMKLAMIKVYQKLKENNLKSKIILQVHDELLI
DPO1_ECOLI   DIKSSNGARRAAAERAAINAPMQGTAADIIKRAMIAVDAWLQAEQPRVRMIMQVHDELVF
              . .     .   ** *.*.*.**.****.* *** .   *.  .  . ..*.******.
```

Fig. 17/3

```
chimera___   EAPYEEKDIVKEIVKREMENAVALKVPLVVEVKEGLNWYENKI
tne.rse___   EVPNEEKDALVELVKDRMTNVVKLSVPLEVDVTIGKTWS----
ath.rse___   EAPYEEKDIVKEIVKREMENAVALKVPLVVEVKEGLNWYENKI
DPO1_ECOLI   EVHKDDVDAVAKQIHQLMENCTRLDVPLLVEVGSGENWDQAH-
             *   .. * .   ..  * *   * *** *.*  * .*
```

Figure 18/1:

SEQ ID No.:. 19
SEQ ID No.: 20
SEQ ID No.: 21
SEQ ID NO.: 22

```
TNE UP    5´   CTG ACC ATG GCG AGA CTA TTT CTC TTT G  -3´
TNE LOW   5´   TCT GTC GAC CTT CAC ACC GTT CAG TTC CAT CC  -3´
ATH UP    5´ - AAG GTC GAC AGA GAT GCC CTC ATC CAA TAT ACC -3´
ATH LOW   5´ - TAG CAA GCT TCT ATT TTG TCT CAT ACC AGT -3´
```

A.

crossing point 1

SEQ ID No.: 23
SEQ ID No.: 24
SEQ ID No.: 25

```
chimera__8   IEMPLVSVLARMELNGV  |  KVDRDALIQYTKEIENKILKLETQIYQIAGEWFNINSPKQLSY
tne.rse__    IEMPLVSVLARMELNGV  |  YVDTEFLKKLSEEYGKKLEELAEEIYRIAGEPFNINSPKQVSR
ath.rse__    IERPLIPVLYEMEKTGF  |  KVDRDALIQYTKEIENKILKLETQIYQIAGEWFNINSPKQLSY
```

B.

SEQ ID No.: 19
SEQ ID No.: 26
SEQ ID No.: 27

```
            5´ ctg acc ATG GCG AGA CTA TTT CTC TTT G -3´
               TNEUP   |---------------------------->
                       ATG GCG AGA CTA TTT CTC TTT GAT GGA    27
                        M   A   R   L   F   L   F   D   G     9

1
 ..............................................................
```

SEQ ID No.: 28
SEQ ID No.: 29
SEQ ID No.: 20
SEQ ID No.: 21
SEQ ID No.: 30
SEQ ID No.: 31

```
1512  CGG ATG GAA CTG AAC GGT GTG TAC GTG GAC ACA GAG TTC CTG AAG AAA CTC      1563
 505   R   M   E   L   N   G   V   Y   V   D   T   E   F   L   K   K   L       521
     3´CC CAT CTT GAC TTG CCA CAC ctt CAg cTG TcT 5´
       <----------------------------==============| TNELOW
                          "Sal I site "
                        ATHUP  |=====================------------------------------->
                               5´  AAG Gtc Gac AGA GAT GCC CTC ATC CAA TAT ACC -3´
1387       ATG GAA AAA ACA GGA TTT AAG GTG GAT AGA GAT GCC CTC ATC CAA TAT ACC  1435
 463        M   E   K   T   G   F   K   V   D   R   D   A   L   I   Q   Y   T   479

 ..............................................................
```

Figure 18/2:

SEQ ID No.: 32
SEQ ID No.: 33
SEQ ID No.: 22

```
2526   GGA CTG AAC TGG TAT GAG ACA AAA TAG                2553
 843    G   L   N   W   Y   E   T   K   *
          3´TG ACC ATA CTC TGT TTT ATC ttcgaacgat 5´
            <-----------------------------------| ATHLOW
```

Figure 19:

SEQ ID No.: 34
SEQ ID No.: 35
SEQ ID No.: 36

```
                                                    crossing point 2
chimera__8    IMEPLVSVLARMELNGVYVDTEFLKKLSEEYGKKLEELAEEIYRIAGEPFNINSPKQVS|R
tne.rse___    IEMPLVSVLARMELNGVYVDTEFLKKLSEEYGKKLEELAEEIYRIAGEPFNINSPKQVS|R
ath.rse___    IERPLIPVLYEMEKTGFKVDRDALIQYTKEIENKILKLETQIYQIAGEWFNINSPKQLS|R
```

A.

TNE polymerase nucleotide sequence 1642-1689

SEQ ID No.: 37
SEQ ID No.: 38

```
                    Bam HI site
                          ========
1642  TCA CCG AAG CAG GTT TCA AGG ATC CTT TTT GAA AAA CTC GGC ATA AAA   1689
 548   S   P   K   Q   V   S   R   I   L   F   E   K   L   G   I   K    563
```

SEQ ID No.: 39
SEQ ID No.: 40
SEQ ID No.: 41
SEQ ID No.: 42

ATH polyerase nucleotide sequence 1513 - 1560

```
1513   TCA CCG AAA CAG CTT TCT TAC ATT TTG TTT GAA AAG CTA AAA CTT CCT  1560
 505    S   P   K   Q   L   S   Y   I   L   F   E   K   L   K   L   P   520

      5´CA CCG AAA CAG CTT TCT agg atc cTG TTT GAA AAG CTA AAA CTT CCT G 3´
       |----------------m1------------------------------------------------>

.....3´GT GGC TTT GTC GAA AGA tcc tag gAC AAA CTT TTC GAT TTT GAA GGA C 5´
      <----------------m2-----------------------------------------------|
```

B.

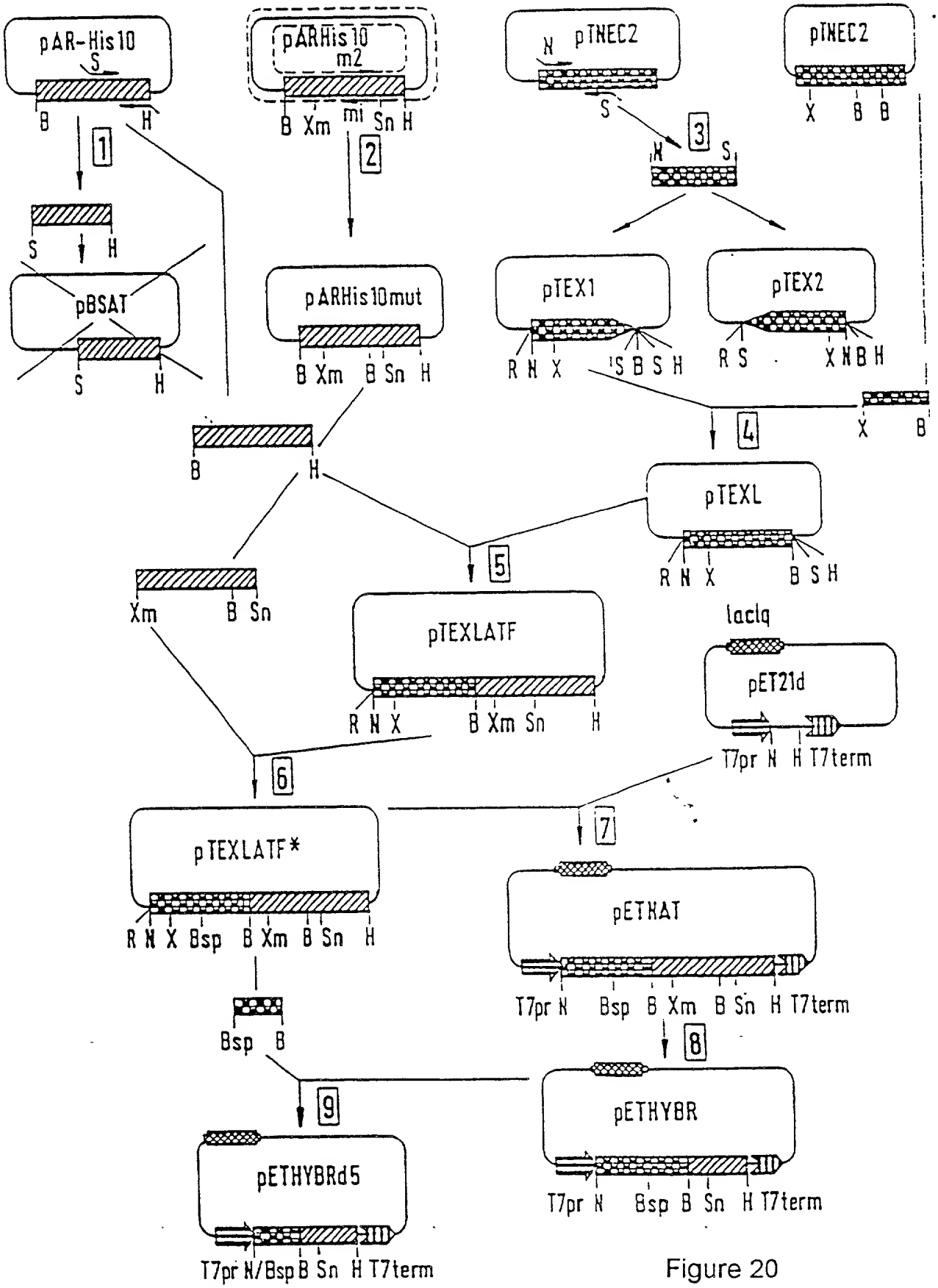

pAR-His10
S
B
H
1
pARHis10 m2
B Xm mi Sn H
2
pTNEC2
N
S
3
pTNEC2
X B B
N S
S H
pBSAT
S H
pARHis10mut
B Xm B Sn H
pTEX1
R N X S B S H
pTEX2
R S X N B H
4
X B
B H
pTEXL
R N X B S H
5
Xm B Sn
pTEXLATF
R N X B Xm Sn H
lacIq
pET21d
T7pr N H T7term
6
7
pTEXLATF*
R N X Bsp B Xm B Sn H
pETHAT
T7pr N Bsp B Xm B Sn H T7term
Bsp B
8
9
pETHYBR
T7pr N Bsp B Sn H T7term
pETHYBRd5
T7pr N/Bsp B Sn H T7term

Figure 20

Figure 21

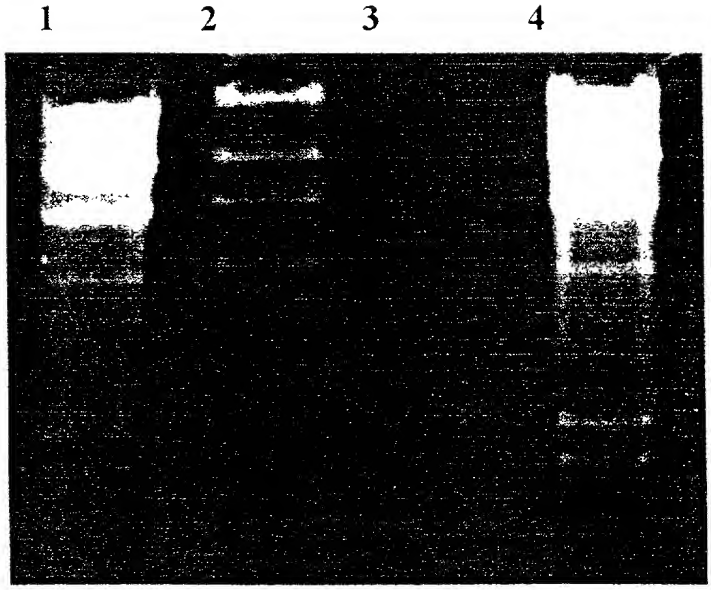
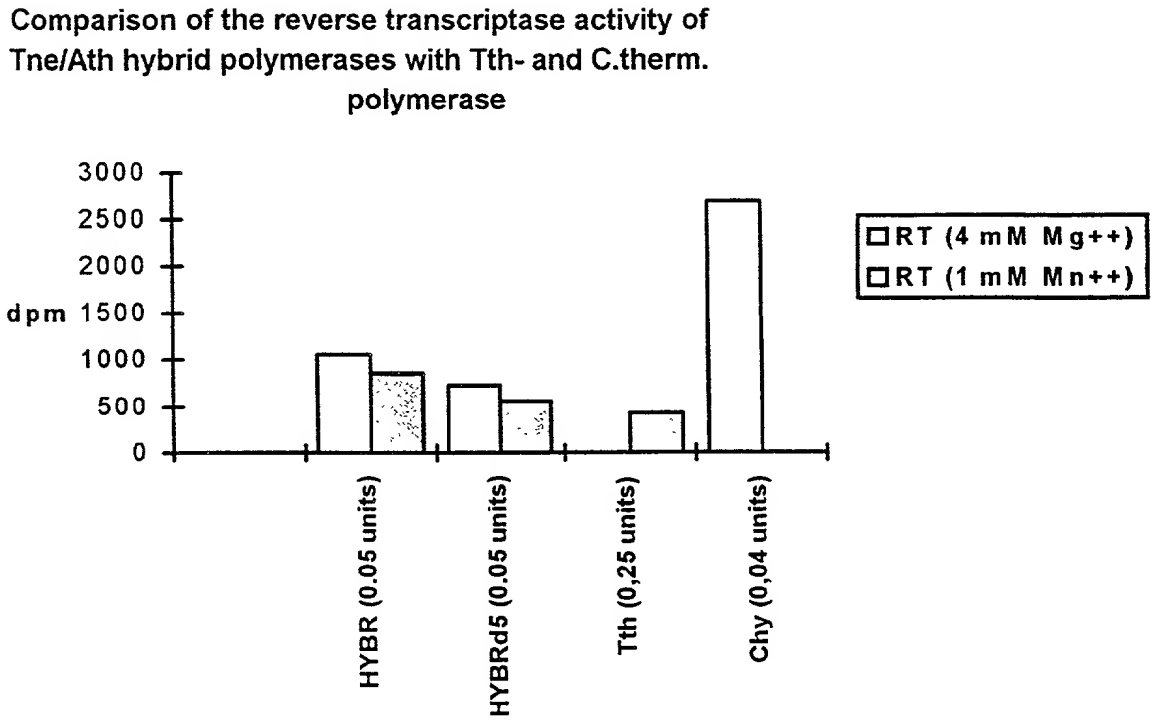


Figure 22:


Comparison of the reverse transcriptase activity of Tne/Ath hybrid polymerases with Tth- and C.therm. polymerase
3000
2500
2000
dpm 1500
1000
500
0
HYBR (0.05 units)
HYBRd5 (0.05 units)
Tth (0,25 units)
Chy (0,04 units)
RT (4 mM Mg++)
RT (1 mM Mn++)

Application Serial No: 09/623,326
Filed on August 30, 2000
Attorney Docket No. 4894

Declaration and Power of Attorney for Patent Application

As the below named inventor(s), We hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

POLYMERASE CHIMERAS

---

the specification of which (check one)

[ ] is attached hereto.

[ ] was filed on _____ as

Application Serial No. _____

and was amended on _____ (if applicable).

[X] was filed on March 15, 1999 as

PCT International Application Serial No. PCT/EP99/01674

and was amended under PCT Article 19 on 14 April 2000 (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			**Priority Claimed**	
198 10 879.6	Germany	13 March 1998	[X]	[ ]
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
_____	_____	_____	[ ]	[ ]
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

(Application No.)	(Filing Date)	(Application No.)	(Filing Date)
(Application No.)	(Filing Date)	(Application No.)	(Filing Date)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s), or §365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

PCT/EP99/01674	15 March 1999	Pending
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the practitioners at customer number 22829 to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

Send Correspondence to: Customer Number 22829

Direct Telephone Calls to: Victor K. Lee, Ph.D.
(510) 814-2966

1 00

BRUNO FREY

Full Name Of Sole Or First Inventor .

Bruno Frey

Sole Or First Inventor's Signature

11/22/00

Date

Penzberg, Germany DEX

Residence

German

Citizenship

Hochfeldstrasse 50, D-82377 Penzberg, Germany

Post Office Address

2 00

BRITTA VILLBRANDT

Full Name Of Second Joint Inventor, If Any

Britta Villbrandt

12/18/00

Villbrandt 12/03/00

Villbrandt

Second Inventor's Signature

Date

Braunschweig, Germany DEX

Residence

German

Citizenship

Fasanenstrasse 19, D-38102 Braunschweig, Germany

Post Office Address

3 D

DIETMAR SCHOMBURG

Full Name Of Third Joint Inventor, If Any

Dietmar Schomburg — 12/03/00

Third Inventor's Signature — Date

Erftstadt, Germany DEX

Residence

German

Citizenship

Richardstrasse 35, D-50374 Erftstadt, Germany

Post Office Address

4 D

HARALD SOBEK

Full Name Of Fourth Joint Inventor, If Any

Harald Sobek — 24. Nov. 00

Fourth Inventor's Signature — Date

Penzberg, Germany DEX

Residence

German

Citizenship

Birkenstrasse 29, D-82377 Penzberg, Germany

Post Office Address

5 WALTRAUD ANKENBAUER
Full Name Of Fifth Joint Inventor, If Any

X Waltraud Ankenbauer
Fifth Inventor's Signature

23.11.00
Date

Penzberg, Germany DEX
Residence

German
Citizenship

Oberanger 18, D-82377, Penzberg, Germany
Post Office Address

Title 37, Code of Federal Regulations, §1.56, duty to disclose information material to patentability provides, in part, that each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is cancelled or withdrawn from consideration, or the application becomes abandoned.

Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and

(1) It establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim; or

(2) It refutes, or is inconsistent with, a position the applicant takes in:
   (i) Opposing an argument of unpatentability relied on by the Office, or
   (ii) Asserting an argument of patentability.

SEQUENCE PROTOCOL

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Boehringer Mannheim GmbH
(B) ROAD: Sandhoferstr. 116
(C) CITY: Mannheim
(E) COUNTRY: DE
(F) POSTAL CODE: 68305
(G) TELEPHONE: 06217595482
(H) TELEFAX: 06217594457

(ii) TITLE OF INVENTION: Polymerase chimeras

(iii) NUMBER OF SEQUENCES: 14

(iv) COMPUTER READABLE FORM:
(A) DATA CARRIER: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 2733 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single strand
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ATGAGGGGCT CGCATCACCA TCACCATCAC GCTGCTGACG ATGACGATAA AATGAGGGGC 60

ATGCTACCGC TATTTGAGCC CAAGGGCCGG GTCCTCCTGG TCGACGGCCA CCACCTGGCC 120

TACCGCACCT TCCACGCCCT GAAGGGCCTC ACCACCAGCC GGGGGGAGCC GGTGCAGGCG 180

GTCTACGGCT TCGCCAAGAG CCTCCTCAAG GCCCTCAAGG AGGACGGGGA CGCGGTGATC 240

GTGGTCTTTG ACGCCAAGGC CCCCTCCTTC CGCCACGAGG CCTACGGGGG GTACAAGGCG 300

GGCCGGGCCC CCACGCCGGA GGACTTTCCC CGGCAACTCG CCCTCATCAA GGAGCTGGTG 360

GACCTCCTGG GGCTGGCGCG CCTCGAGGTC CCGGGCTACG AGGCGGACGA CGTCCTGGCC 420

AGCCTGGCCA AGAAGGCGGA AAAGGAGGGC TACGAGGTCC GCATCCTCAC CGCCGACAAA 480

GACCTTTACC AGCTCCTTTC CGACCGCATC CACGTCCTCC ACCCCGAGGG GTACCTCATC 540

ACCCCGGCCT GGCTTTGGGA AAAGTACGGC CTGAGGCCCG ACCAGTGGGC CGACTACCGG 600

GCCCTGACCG GGGACGAGTC CGACAACCTT CCCGGGGTCA AGGGCATCGG GGAGAAGACG 660

GCGAGGAAGC TTCTGGAGGA GTGGGGGAGC CTGGAAGCCC TCCTCAAGAA CCTGGACCGG 720

CTGAAGCCCG CCATCCGGGA GAAGATCCTG GCCCACATGG ACGATCTGAA GCTCTCCTGG 780

GACCTGGCCA AGGTGCGCAC CGACCTGCCC CTGGAGGTGG ACTTCGCCAA AAGGCGGGAG 840

CCCGACCGGG AGAGGCTTAG GGCCTTTCTG GAGAGGCTTG AGTTTGGCAG CCTCCTCCAC 900

GAGTTCGGCC TTCTGGAAAG CCCCTATGAC AACTACGTCA CCATCCTTGA TGAAGAAACA 960

CTGAAAGCGT GGATTGCGAA GCTGGAAAAA GCGCCGGTAT TTGCATTTGA TACCGAAACC 1020

GACAGCCTTG ATAACATCTC TGCTAACCTG GTCGGGCTTT CTTTTGCTAT CGAGCCAGGC 1080

GTAGCGGCAT ATATTCCGGT TGCTCATGAT TATCTTGATG CGCCCGATCA AATCTCTCGC 1140

GAGCGTGCAC TCGAGTTGCT AAAACCGCTG CTGGAAGATG AAAAGGCGCT GAAGGTCGGG 1200

CAAAACCTGA AATACGATCG CGGTATTCTG GCGAACTACG GCATTGAACT GCGTGGGATT 1260

GCGTTTGATA CCATGCTGGA GTCCTACATT CTCAATAGCG TTGCCGGGCG TCACGATATG 1320

GACAGCCTCG CGGAACGTTG GTTGAAGCAC AAAACCATCA CTTTTGAAGA GATTGCTGGT 1380

AAAGGCAAAA ATCAACTGAC CTTTAACCAG ATTGCCCTCG AAGAAGCCGG ACGTTACGCC 1440

GCCGAAGATG CAGATGTCAC CTTGCAGTTG CATCTGAAAA TGTGGCCGGA TCTGCAAAAA 1500

CACGAGAGGC TCCTTTGGCT TTACCGGGAG GTGGAGAGGC CCCTTTCCGC TGTCCTGGCC 1560

CACATGGAGG CCACGGGGGT GCGCCTGGAC GTGGCCTATC TCAGGGCCTT GTCCCTGGAG 1620

GTGGCCGAGG AGGTCGCCCG CCTCGAGGCC GAGGTCTTCC GCCTGGCCGG CCACCCCTTC 1680

AACCTCAACT CCCGGGACCA GCTGGAAAGG GTCCTCTTTG ACGAGCTAGG GCTTCCCGCC 1740

ATCGGCAAGA CGGAGAAGAC CGGCAAGCGC TCCACCAGCG CCGCCGTCCT GGAGGCCCTC 1800

CGCGAGGCCC ACCCCATCGT GGAGAAGATC CTGCAGTACC GGGAGCTCAC CAAGCTGAAG 1860

AGCACCTACA TTGACCCCTT GCCGGACCTC ATCCACCCCA GGACGGGCCG CCTCCACACC 1920

CGCTTCAACC AGACGGCCAC GGCCACGGGC AGGCTAAGTA GCTCCGATCC CAACCTCCAG 1980

AACATCCCCG TCCGCACCCC GCTTGGGCAG AGGATCCGCC GGGCCTTCAT CGCCGAGGAG 2040

GGGTGGCTAT TGGTGGCCCT GGACTATAGC CAGATAGAGC TCAGGGTGCT GGCCCACCTC 2100

TCCGGCGACG AGAACCTGAT CCGGGTCTTC CAGGAGGGGC GGGACATCCA CACGGAGACC 2160

GCCAGCTGGA TGTTCGGCGT CCCCCGGGAG GCCGTGGACC CCCTGATGCG CCGGGCGGCC 2220

AAGACCATCA ACTTCGGGGT CCTCTACGGC ATGTCGGCCC ACCGCCTCTC CCAGGAGCTA 2280

GCCATCCCTT ACGAGGAGGC CCAGGCCTTC ATTGAGCGCT ACTTTCAGAG CTTCCCCAAG 2340

GTGCGGGCCT GGATTGAGAA GACCCTGGAG GAGGGCAGGA GGCGGGGGTA CGTGGAGACC 2400

CTCTTCGGCC GCCGCCGCTA CGTGCCAGAC CTAGAGGCCC GGGTGAAGAG CGTGCGGGAG 2460

GCGGCCGAGC GCATGGCCTT CAACATGCCC GTCCAGGGCA CCGCCGCCGA CCTCATGAAG 2520

CTGGCTATGG TGAAGCTCTT CCCCAGGCTG GAGGAAATGG GGGCCAGGAT GCTCCTTCAG 2580

GTCCACGACG AGCTGGTCCT CGAGGCCCCA AAAGAGAGGG CGGAGGCCGT GGCCCGGCTG 2640

GCCAAGGAGG TCATGGAGGG GGTGTATCCC CTGGCCGTGC CCCTGGAGGT GGAGGTGGGG 2700

ATAGGGGAGG ACTGGCTCTC CGCCAAGGAG TGA 2733

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 2733 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single strand
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ATGAGGGGCT CGCATCACCA TCACCATCAC GCTGCTGACG ATGACGATAA AATGAGGGGC 60

ATGCTACCGC TATTTGAGCC CAAGGGCCGG GTCCTCCTGG TCGACGGCCA CCACCTGGCC 120

TACCGCACCT TCCACGCCCT GAAGGGCCTC ACCACCAGCC GGGGGGAGCC GGTGCAGGCG 180

GTCTACGGCT TCGCCAAGAG CCTCCTCAAG GCCCTCAAGG AGGACGGGGA CGCGGTGATC 240

GTGGTCTTTG ACGCCAAGGC CCCCTCCTTC CGCCACGAGG CCTACGGGGG GTACAAGGCG 300

GGCCGGGCCC CCACGCCGGA GGACTTTCCC CGGCAACTCG CCCTCATCAA GGAGCTGGTG 360

GACCTCCTGG GGCTGGCGCG CCTCGAGGTC CCGGGCTACG AGGCGGACGA CGTCCTGGCC 420

AGCCTGGCCA AGAAGGCGGA AAAGGAGGGC TACGAGGTCC GCATCCTCAC CGCCGACAAA 480

GACCTTTACC AGCTCCTTTC CGACCGCATC CACGTCCTCC ACCCCGAGGG GTACCTCATC 540

ACCCCGGCCT GGCTTTGGGA AAAGTACGGC CTGAGGCCCG ACCAGTGGGC CGACTACCGG 600

GCCCTGACCG GGGACGAGTC CGACAACCTT CCCGGGGTCA AGGGCATCGG GGAGAAGACG 660

GCGAGGAAGC TTCTGGAGGA GTGGGGGAGC CTGGAAGCCC TCCTCAAGAA CCTGGACCGG 720

CTGAAGCCCG CCATCCGGGA GAAGATCCTG GCCCACATGG ACGATCTGAA GCTCTCCTGG 780

GACCTGGCCA AGGTGCGCAC CGACCTGCCC CTGGAGGTGG ACTTCGCCAA AAGGCGGGAG 840

CCCGACCGGG AGAGGCTTAG GGCCTTTCTG GAGAGGCTTG AGTTTGGCAG CCTCCTCCAC 900

GAGTTCGGCC TTCTGGAAAG CCCCTATGAC AACTACGTCA CCATCCTTGA TGAAGAAACA 960

CTGAAAGCGT GGATTGCGAA GCTGGAAAAA GCGCCGGTAT TTGCATTTGA TACCGAAACC 1020

GACAGCCTTG ATAACATCTC TGCTAACCTG GTCGGGCTTT CTTTTGCTAT CGAGCCAGGC 1080

GTAGCGGCAT ATATTCCGGT TGCTCATGAT TATCTTGATG CGCCCGATCA AATCTCTCGC 1140

GAGCGTGCAC TCGAGTTGCT AAAACCGCTG CTGGAAGATG AAAAGGCGCT GAAGGTCGGG 1200

CAAAACCTGA AATACGATCG CGGTATTCTG GCGAACTACG GCATTGAACT GCGTGGGATT 1260

GCGTTTGATA CCATGCTGGA GTCCTACATT CTCAATAGCG TTGCCGGGCG TCACGATATG 1320

GACAGCCTCG CGGAACGTTG GTTGAAGCAC AAAACCATCA CTTTTGAAGA GATTGCTGGT 1380

AAAGGCAAAA ATCAACTGAC CTTTAACCAG ATTGCCCTCG AAGAAGCCGG ACGTTACGCC 1440

GCCGAAGATG CAGATGTCAC CTTGCAGTTG CATCTGAAAA TGTGGCCGGA TCTGCAAAAA 1500

CACAAAGGGC CGTTGAACGT CTTCGAGAAT ATCGAAATGC CGCTGGTGCC GGTGCTTTCA 1560

CGCATTGAAC GTAACGGTGT GCGCCTGGAC GTGGCCTATC TCAGGGCCTT GTCCCTGGAG 1620

GTGGCCGAGG AGATCGCCCG CCTCGAGGCC GAGGTCTTCC GCCTGGCCGG CCACCCCTTC 1680

AACCTCAACT CCCGGGACCA GCTGGAAAGG GTCCTCTTTG ACGAGCTAGG GCTTCCCGCC 1740

ATCGGCAAGA CGGAGAAGAC CGGCAAGCGC TCCACCAGCG CCGCCGTCCT GGAGGCCCTC 1800

CGCGAGGCCC ACCCCATCGT GGAGAAGATC CTGCAGTACC GGGAGCTCAC CAAGCTGAAG 1860

AGCACCTACA TTGACCCCTT GCCGGACCTC ATCCACCCCA GGACGGGCCG CCTCCACACC 1920

CGCTTCAACC AGACGGCCAC GGCCACGGGC AGGCTAAGTA GCTCCGATCC CAACCTCCAG 1980

AACATCCCCG TCCGCACCCC GCTTGGGCAG AGGATCCGCC GGGCCTTCAT CGCCGAGGAG 2040

GGGTGGCTAT TGGTGGCCCT GGACTATAGC CAGATAGAGC TCAGGGTGCT GGCCCACCTC 2100

TCCGGCGACG AGAACCTGAT CCGGGTCTTC CAGGAGGGGC GGGACATCCA CACGGAGACC 2160

GCCAGCTGGA TGTTCGGCGT CCCCCGGGAG GCCGTGGACC CCCTGATGCG CCGGGCGGCC 2220

AAGACCATCA ACTTCGGGGT CCTCTACGGC ATGTCGGCCC ACCGCCTCTC CCAGGAGCTA 2280

GCCATCCCTT ACGAGGAGGC CCAGGCCTTC ATTGAGCGCT ACTTTCAGAG CTTCCCCAAG 2340

GTGCGGGCCT GGATTGAGAA GACCCTGGAG GAGGGCAGGA GGCGGGGGTA CGTGGAGACC 2400

CTCTTCGGCC GCCGCCGCTA CGTGCCAGAC CTAGAGGCCC GGGTGAAGAG CGTGCGGGAG 2460

GCGGCCGAGC GCATGGCCTT CAACATGCCC GTCCAGGGCA CCGCCGCCGA CCTCATGAAG 2520

CTGGCTATGG TGAAGCTCTT CCCCAGGCTG GAGGAAATGG GGGCCAGGAT GCTCCTTCAG 2580

GTCCACGACG AGCTGGTCCT CGAGGCCCCA AAAGAGAGGG CGGAGGCCGT GGCCCGGCTG 2640

GCCAAGGAGG TCATGGAGGG GGTGTATCCC CTGGCCGTGC CCCTGGAGGT GGAGGTGGGG 2700

ATAGGGGAGG ACTGGCTCTC CGCCAAGGAG TGA 2733

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2727 base pairs
(B) Type: nucleotide
(C) STRANDEDNESS: single strand
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATGAGGGGCT CGCATCACCA TCACCATCAC GCTGCTGACG ATGACGATAA AATGAGGGGC 60

ATGCTACCGC TATTTGAGCC CAAGGGCCGG GTCCTCCTGG TCGACGGCCA CCACCTGGCC 120

TACCGCACCT TCCACGCCCT GAAGGGCCTC ACCACCAGCC GGGGGGAGCC GGTGCAGGCG 180

GTCTACGGCT TCGCCAAGAG CCTCCTCAAG GCCCTCAAGG AGGACGGGGA CGCGGTGATC 240

GTGGTCTTTG ACGCCAAGGC CCCCTCCTTC CGCCACGAGG CCTACGGGGG GTACAAGGCG 300

GGCCGGGCCC CCACGCCGGA GGACTTTCCC CGGCAACTCG CCCTCATCAA GGAGCTGGTG 360

GACCTCCTGG GGCTGGCGCG CCTCGAGGTC CCGGGCTACG AGGCGGACGA CGTCCTGGCC 420

AGCCTGGCCA AGAAGGCGGA AAAGGAGGGC TACGAGGTCC GCATCCTCAC CGCCGACAAA 480

GACCTTTACC AGCTCCTTTC CGACCGCATC CACGTCCTCC ACCCCGAGGG GTACCTCATC 540

ACCCCGGCCT GGCTTTGGGA AAAGTACGGC CTGAGGCCCG ACCAGTGGGC CGACTACCGG 600

GCCCTGACCG GGGACGAGTC CGACAACCTT CCCGGGGTCA AGGGCATCGG GGAGAAGACG 660

GCGAGGAAGC TTCTGGAGGA GTGGGGGAGC CTGGAAGCCC TCCTCAAGAA CCTGGACCGG 720

CTGAAGCCCG CCATCCGGGA GAAGATCCTG GCCCACATGG ACGATCTGAA GCTCTCCTGG 780

GACCTGGCCA AGGTGCGCAC CGACCTGCCC CTGGAGGTGG ACTTCGCCAA AAGGCGGGAG 840

CCCGACCGGG AGAGGCTTAG GGCCTTTCTG GAGAGGCTTG AGTTTGGCAG CCTCCTCCAC 900

GAGTTCGGCC TTCTGGAAAG CCCCCCCGTT GGATACAGAA TAGTGAAAGA CCTGGTGGAA 960

TTTGAAAAAC TCATAGAGAA ACTGAGAGAA TCCCCTTCGT TCGCCATAGA TCTTGAGACG 1020

TCTTCCCTCG ATCCTTTCGA CTGCGACATT GTCGGTATCT CTGTGTCTTT CAAACCAAAG 1080

GAAGCGTACT ACATACCACT CCATCATAGA AACGCCCAGA ACCTGGATGA AAAAGAAGTT 1140

CTGAAAAAGC TAAAAGAAAT CCTGGAGGAC CCCGGAGCAA AGATCGTTGG TCAGAATTTG 1200

AAATTCGATT ACAAGGTGTT GATGGTAAAG GGTGTTGAAC CTGTCCCTCC TCACTTCGAC 1260

ACGATGATAG CGGCTTACCT TCTTGAGCCG AACGAAAAGA AGTTCAATCT GGACGATCTC 1320

GCATTGAAAT TTCTTGGATA CAAAATGACC TCTTACCAGG AACTCATGTC CTTCTCTTCT 1380

CCGCTGTTTG GTTTCAGTTT TGCCGATGTT CCTGTAGAAA AAGCAGCGAA CTATTCCTGT 1440

GAAGATGCCG ACATCACCTA CAGACTCTAC AAGATCCTGA GCTTAAAACT CCACGAGGAG 1500

AGGCTCCTTT GGCTTTACCG GGAGGTGGAG AGGCCCCTTT CCGCTGTCCT GGCCCACATG 1560

GAGGCCACGG GGGTGCGCCT GGACGTGGCC TATCTCAGGG CCTTGTCCCT GGAGGTGGCC 1620

GAGGAGATCG CCCGCCTCGA GGCCGAGGTC TTCCGCCTGG CCGGCCACCC CTTCAACCTC 1680

AACTCCCGGG ACCAGCTGGA AAGGGTCCTC TTTGACGAGC TAGGGCTTCC CGCCATCGGC 1740

AAGACGGAGA AGACCGGCAA GCGCTCCACC AGCGCCGCCG TCCTGGAGGC CCTCCGCGAG 1800

GCCCACCCCA TCGTGGAGAA GATCCTGCAG TACCGGGAGC TCACCAAGCT GAAGAGCACC 1860

TACATTGACC CCTTGCCGGA CCTCATCCAC CCCAGGACGG GCCGCCTCCA CACCCGCTTC 1920

AACCAGACGG CCACGGCCAC GGGCAGGCTA AGTAGCTCCG ATCCCAACCT CCAGAACATC 1980

CCCGTCCGCA CCCCGCTTGG GCAGAGGATC CGCCGGGCCT TCATCGCCGA GGAGGGGTGG 2040

CTATTGGTGG CCCTGGACTA TAGCCAGATA GAGCTCAGGG TGCTGGCCCA CCTCTCCGGC 2100

GACGAGAACC TGATCCGGGT CTTCCAGGAG GGGCGGGACA TCCACACGGA GACCGCCAGC 2160

TGGATGTTCG GCGTCCCCCG GGAGGCCGTG GACCCCCTGA TGCGCCGGGC GGCCAAGACC 2220

ATCAACTTCG GGGTCCTCTA CGGCATGTCG GCCCACCGCC TCTCCCAGGA GCTAGCCATC 2280

CCTTACGAGG AGGCCCAGGC CTTCATTGAG CGCTACTTTC AGAGCTTCCC CAAGGTGCGG 2340

GCCTGGATTG AGAAGACCCT GGAGGAGGGC AGGAGGCGGG GGTACGTGGA GACCCTCTTC 2400

GGCCGCCGCC GCTACGTGCC AGACCTAGAG GCCCGGGTGA AGAGCGTGCG GGAGGCGGCC 2460

GAGCGCATGG CCTTCAACAT GCCCGTCCAG GGCACCGCCG CCGACCTCAT GAAGCTGGCT 2520

ATGGTGAAGC TCTTCCCCAG GCTGGAGGAA ATGGGGGCCA GGATGCTCCT TCAGGTCCAC 2580

GACGAGCTGG TCCTCGAGGC CCCAAAAGAG AGGGCGGAGG CCGTGGCCCG GCTGGCCAAG 2640

GAGGTCATGG AGGGGGTGTA TCCCCTGGCC GTGCCCCTGG AGGTGGAGGT GGGGATAGGG 2700

GAGGACTGGC TCTCCGCCAA GGAGTGA 2727

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2727 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single strand
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

ATGAGGGGCT CGCATCACCA TCACCATCAC GCTGCTGACG ATGACGATAA AATGAGGGGC 60

ATGCTACCGC TATTTGAGCC CAAGGGCCGG GTCCTCCTGG TCGACGGCCA CCACCTGGCC 120

TACCGCACCT TCCACGCCCT GAAGGGCCTC ACCACCAGCC GGGGGGAGCC GGTGCAGGCG 180

GTCTACGGCT TCGCCAAGAG CCTCCTCAAG GCCCTCAAGG AGGACGGGGA CGCGGTGATC 240

GTGGTCTTTG ACGCCAAGGC CCCCTCCTTC CGCCACGAGG CCTACGGGGG GTACAAGGCG 300

GGCCGGGCCC CCACGCCGGA GGACTTTCCC CGGCAACTCG CCCTCATCAA GGAGCTGGTG 360

GACCTCCTGG GGCTGGCGCG CCTCGAGGTC CCGGGCTACG AGGCGGACGA CGTCCTGGCC 420

AGCCTGGCCA AGAAGGCGGA AAAGGAGGGC TACGAGGTCC GCATCCTCAC CGCCGACAAA 480

GACCTTTACC AGCTCCTTTC CGACCGCATC CACGTCCTCC ACCCCGAGGG GTACCTCATC 540

ACCCCGGCCT GGCTTTGGGA AAAGTACGGC CTGAGGCCCG ACCAGTGGGC CGACTACCGG 600

GCCCTGACCG GGGACGAGTC CGACAACCTT CCCGGGGTCA AGGGCATCGG GGAGAAGACG 660

GCGAGGAAGC TTCTGGAGGA GTGGGGGAGC CTGGAAGCCC TCCTCAAGAA CCTGGACCGG 720

CTGAAGCCCG CCATCCGGGA GAAGATCCTG GCCCACATGG ACGATCTGAA GCTCTCCTGG 780

GACCTGGCCA AGGTGCGCAC CGACCTGCCC CTGGAGGTGG ACTTCGCCAA AAGGCGGGAG 840

CCCGACCGGG AGAGGCTTAG GGCCTTTCTG GAGAGGCTTG AGTTTGGCAG CCTCCTCCAC 900

GAGTTCGGCC TTCTGGAAAG CCCCCCCGTT GGATACAGAA TAGTGAAAGA CCTGGTGGAA 960

TTTGAAAAAC TCATAGAGAA ACTGAGAGAA TCCCCTTCGT TCGCCATAGA TCTTGAGACG 1020

TCTTCCCTCG ATCCTTTCGA CTGCGACATT GTCGGTATCT CTGTGTCTTT CAAACCAAAG 1080

GAAGCGTACT ACATACCACT CCATCATAGA AACGCCCAGA ACCTGGATGA AAAAGAAGTT 1140

CTGAAAAAGC TAAAAGAAAT CCTGGAGGAC CCCGGAGCAA AGATCGTTGG TCAGAATTTG 1200

AAATTCGATT ACAAGGTGTT GATGGTAAAG GGTGTTGAAC CTGTCCCTCC TCACTTCGAC 1260

ACGATGATAG CGGCTTACCT TCTTGAGCCG AACGAAAAGA AGTTCAATCT GGACGATCTC 1320

GCATTGAAAT TTCTTGGATA CAAAATGACC TCTTACCAGG AACTCATGTC CTTCTCTTCT 1380

CCGCTGTTTG GTTTCAGTTT TGCCGATGTT CCTGTAGAAA AAGCAGCGAA CTATTCCTGT 1440

GAAGATGCAG ACATCACCTA CAGACTCTAC AAGATCCTGA GCTTAAAACT CCACGAGGCA 1500

GATCTGGAGA ACGTGTTCTA CAAGATAGAA ATGCCTCTTG TGAGCGTGCT TGCACGGATG 1560

GAACTGAACG GTGTGCGCCT GGACGTGGCC TATCTCAGGG CCTTGTCCCT GGAGGTGGCC 1620

GAGGAGATCG CCCGCCTCGA GGCCGAGGTC TTCCGCCTGG CCGGCCACCC CTTCAACCTC 1680

AACTCCCGGG ACCAGCTGGA AAGGGTCCTC TTTGACGAGC TAGGGCTTCC CGCCATCGGC 1740

AAGACGGAGA AGACCGGCAA GCGCTCTACC AGCGCCGCCG TCCTGGAGGC CCTCCGCGAG 1800

GCCCACCCCA TCGTGGAGAA GATCCTGCAG TACCGGGAGC TCACCAAGCT GAAGAGCACC 1860

TACATTGACC CCTTGCCGGA CCTCATCCAC CCCAGGACGG GCCGCCTCCA CACCCGCTTC 1920

AACCAGACGG CCACGGCCAC GGGCAGGCTA AGTAGCTCCG ATCCCAACCT CCAGAACATC 1980

CCCGTCCGCA CCCCGCTTGG GCAGAGGATC CGCCGGGCCT TCATCGCCGA GGAGGGGTGG 2040

CTATTGGTGG CCCTGGACTA TAGCCAGATA GAGCTCAGGG TGCTGGCCCA CCTCTCCGGC 2100

GACGAGAACC TGATCCGGGT CTTCCAGGAG GGGCGGGACA TCCACACGGA GACCGCCAGC 2160

TGGATGTTCG GCGTCCCCCG GGAGGCCGTG GACCCCCTGA TGCGCCGGGC GGCCAAGACC 2220

ATCAACTTCG GGGTCCTCTA CGGCATGTCG GCCCACCGCC TCTCCCAGGA GCTAGCCATC 2280

CCTTACGAGG AGGCCCAGGC CTTCATTGAG CGCTACTTTC AGAGCTTCCC CAAGGTGCGG 2340

GCCTGGATTG AGAAGACCCT GGAGGAGGGC AGGAGGCGGG GGTACGTGGA GACCCTCTTC 2400

GGCCGCCGCC GCTACGTGCC AGACCTAGAG GCCCGGGTGA AGAGCGTGCG GGAGGCGGCC 2460

GAGCGCATGG CCTTCAACAT GCCCGTCCAG GGCACCGCCG CCGACCTCAT GAAGCTGGCT 2520

ATGGTGAAGC TCTTCCCCAG GCTGGAGGAA ATGGGGGCCA GGATGCTCCT TCAGGTCCAC 2580

GACGAGCTGG TCCTCGAGGC CCCAAAAGAG AGGGCGGAGG CCGTGGCCCG GCTGGCCAAG 2640

GAGGTCATGG AGGGGGTGTA TCCCCTGGCC GTGCCCCTGG AGGTGGAGGT GGGGATAGGG 2700

GAGGACTGGC TCTCCGCCAA GGAGTGA 2727

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2850 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single strand
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULS: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

ATGAGGGGCT CGCATCACCA TCACCATCAC GCTGCTGACG ATGACGATAA AATGAGGGGC 60

ATGCTACCGC TATTTGAGCC CAAGGGCCGG GTCCTCCTGG TCGACGGCCA CCACCTGGCC 120

TACCGCACCT TCCACGCCCT GAAGGGCCTC ACCACCAGCC GGGGGGAGCC GGTGCAGGCG 180

GTCTACGGCT TCGCCAAGAG CCTCCTCAAG GCCCTCAAGG AGGACGGGGA CGCGGTGATC 240

GTGGTCTTTG ACGCCAAGGC CCCCTCCTTC CGCCACGAGG CCTACGGGGG GTACAAGGCG 300

GGCCGGGCCC CCACGCCGGA GGACTTTCCC CGGCAACTCG CCCTCATCAA GGAGCTGGTG 360

GACCTCCTGG GGCTGGCGCG CCTCGAGGTC CCGGGCTACG AGGCGGACGA CGTCCTGGCC 420

AGCCTGGCCA AGAAGGCGGA AAAGGAGGGC TACGAGGTCC GCATCCTCAC CGCCGACAAA 480

GACCTTTACC AGCTCCTTTC CGACCGCATC CACGTCCTCC ACCCCGAGGG GTACCTCATC 540

ACCCCGGCCT GGCTTTGGGA AAAGTACGGC CTGAGGCCCG ACCAGTGGGC CGACTACCGG 600

GCCCTGACCG GGGACGAGTC CGACAACCTT CCCGGGGTCA AGGGCATCGG GGAGAAGACG 660

GCGAGGAAGC TTCTGGAGGA GTGGGGGAGC CTGGAAGCCC TCCTCAAGAA CCTGGACCGG 720

CTGAAGCCCG CCATCCGGGA GAAGATCCTG GCCCACATGG ACGATCTGAA GCTCTCCTGG 780

GACCTGGCCA AGGTGCGCAC CGACCTGCCC CTGGAGGTGG ACTTCGCCAA AAGGCGGGAG 840

CCCGACCGGG AGAGGCTTAG GGCCTTTCTG GAGAGGCTTG AGTTTGGCAG CCTCCTCCAC 900

GAGTTCGGCC TTCTGGAAAG CCCCCATCCA GCAGTTGTGG ACATCTTCGA ATACGATATT 960

CCATTTGCAA AGAGATACCT CATCGACAAA GGCCTAATAC CAATGGAGGG GGAAGAAGAG 1020

CTAAAGATTC TTGCCTTCGA TATAGAAACC CTCTATCACG AAGGAGAAGA GTTTGGAAAA 1080

GGCCCAATTA TAATGATTAG TTATGCAGAT GAAAATGAAG CAAAGGTGAT TACTTGGAAA 1140

AACATAGATC TTCCATACGT TGAGGTTGTA TCAAGCGAGA GAGAGATGAT AAAGAGATTT 1200

CTCAGGATTA TCAGGGAGAA GGATCCTGAC ATTATAGTTA CTTATAATGG AGACTCATTC 1260

GACTTCCCAT ATTTAGCGAA AAGGGCAGAA AAACTTGGGA TTAAATTAAC CATTGGAAGA 1320

GATGGAAGCG AGCCCAAGAT GCAGAGAATA GGCGATATGA CGGCTGTAGA AGTCAAGGGA 1380

AGAATACATT TCGACTTGTA TCATGTAATA ACAAGGACAA TAAATCTCCC AACATACACA 1440

CTAGAGGCTG TATATGAAGC AATTTTTGGA AAGCCAAAGG AGAAGGTATA CGCCGACGAG 1500

ATAGCAAAAG CCTGGGAAAG TGGAGAGAAC CTTGAGAGAG TTGCCAAATA CTCGATGGAA 1560

GATGCAAAGG CAACTTATGA ACTCGGGAAA GAATTCCTTC CAATGGAAAT TCAGCTTTCA 1620

GAGAGGCTCC TTTGGCTTTA CCGGGAGGTG GAGAGGCCCC TTTCCGCTGT CCTGGCCCAC 1680

ATGGAGGCCA CGGGGGTGCG CCTGGACGTG GCCTATCTCA GGGCCTTGTC CCTGGAGGTG 1740

GCCGAGGAGA TCGCCCGCCT CGAGGCCGAG GTCTTCCGCC TGGCCGGCCA CCCCTTCAAC 1800

CTCAACTCCC GGGACCAGCT GGAAAGGGTC CTCTTTGACG AGCTAGGGCT TCCCGCCATC 1860

GGCAAGACGG AGAAGACCGG CAAGCGCTCC ACCAGCGCCG CCGTCCTGGA GGCCCTCCGC 1920

GAGGCCCACC CCATCGTGGA GAAGATCCTG CAGTACCGGG AGCTCACCAA GCTGAAGAGC 1980

ACCTACATTG ACCCCTTGCC GGACCTCATC CACCCCAGGA CGGGCCGCCT CCACACCCGC 2040

TTCAACCAGA CGGCCACGGC CACGGGCAGG CTAAGTAGCT CCGATCCCAA CCTCCAGAAC 2100

ATCCCCGTCC GCACCCCGCT TGGGCAGAGG ATCCGCCGGG CCTTCATCGC CGAGGAGGGG 2160

TGGCTATTGG TGGCCCTGGA CTATAGCCAG ATAGAGCTCA GGGTGCTGGC CCACCTCTCC 2220

GGCGACGAGA ACCTGATCCG GGTCTTCCAG GAGGGGCGGG ACATCCACAC GGAGACCGCC 2280

AGCTGGATGT TCGGCGTCCC CCGGGAGGCC GTGGACCCCC TGATGCGCCG GGCGGCCAAG 2340

ACCATCAACT TCGGGGTCCT CTACGGCATG TCGGCCCACC GCCTCTCCCA GGAGCTAGCC 2400

ATCCCTTACG AGGAGGCCCA GGCCTTCATT GAGCGCTACT TTCAGAGCTT CCCCAAGGTG 2460

CGGGCCTGGA TTGAGAAGAC CCTGGAGGAG GGCAGGAGGC GGGGGTACGT GGAGACCCTC 2520

TTCGGCCGCC GCCGCTACGT GCCAGACCTA GAGGCCCGGG TGAAGAGCGT GCGGGAGGCG 2580

GCCGAGCGCA TGGCCTTCAA CATGCCCGTC CAGGGCACCG CCGCCGACCT CATGAAGCTG 2640

GCTATGGTGA AGCTCTTCCC CAGGCTGGAG GAAATGGGGG CCAGGATGCT CCTTCAGGTC 2700

CACGACGAGC TGGTCCTCGA GGCCCCAAAA GAGAGGGCGG AGGCCGTGGC CCGGCTGGCC 2760

AAGGAGGTCA TGGAGGGGGT GTATCCCCTG GCCGTGCCCC TGGAGGTGGA GGTGGGGATA 2820

GGGGAGGACT GGCTCTCCGC CAAGGAGTGA 2850

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2949 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single strand
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ATGAGGGGCT CGCATCACCA TCACCATCAC GCTGCTGACG ATGACGATAA AATGAGGGGC 60

ATGCTACCGC TATTTGAGCC CAAGGGCCGG GTCCTCCTGG TCGACGGCCA CCACCTGGCC 120

TACCGCACCT TCCACGCCCT GAAGGGCCTC ACCACCAGCC GGGGGGAGCC GGTGCAGGCG 180

GTCTACGGCT TCGCCAAGAG CCTCCTCAAG GCCCTCAAGG AGGACGGGGA CGCGGTGATC 240

GTGGTCTTTG ACGCCAAGGC CCCCTCCTTC CGCCACGAGG CCTACGGGGG GTACAAGGCG 300

GGCCGGGCCC CCACGCCGGA GGACTTTCCC CGGCAACTCG CCCTCATCAA GGAGCTGGTG 360

GACCTCCTGG GGCTGGCGCG CCTCGAGGTC CCGGGCTACG AGGCGGACGA CGTCCTGGCC 420

AGCCTGGCCA AGAAGGCGGA AAAGGAGGGC TACGAGGTCC GCATCCTCAC CGCCGACAAA 480

GACCTTTACC AGCTCCTTTC CGACCGCATC CACGTCCTCC ACCCCGAGGG GTACCTCATC 540

ACCCCGGCCT GGCTTTGGGA AAAGTACGGC CTGAGGCCCG ACCAGTGGGC CGACTACCGG 600

GCCCTGACCG GGGACGAGTC CGACAACCTT CCCGGGGTCA AGGGCATCGG GGAGAAGACG 660

GCGAGGAAGC TTCTGGAGGA GTGGGGGAGC CTGGAAGCCC TCCTCAAGAA CCTGGACCGG 720

CTGAAGCCCG CCATCCGGGA GAAGATCCTG GCCCACATGG ACGATCTGAA GCTCTCCTGG 780

GACCTGGCCA AGGTGCGCAC CGACCTGCCC CTGGAGGTGG ACTTCGCCAA AAGGCGGGAG 840

CCCGACCGGG AGAGGCTTAG GGCCTTTCTG GAGAGGCTTG AGTTTGGCAG CCTCCTCCAC 900

GAGTTCGGCC TTCTGGAAAG CCCCGTTAGA GAACATCCAG CAGTTGTGGA CATCTTCGAA 960

TACGATATTC CATTTGCAAA GAGATACCTC ATCGACAAAG GCCTAATACC AATGGAGGGG 1020

GAAGAAGAGC TAAAGATTCT TGCCTTCGAT ATAGAAACCC TCTATCACGA AGGAGAAGAG 1080

TTTGGAAAAG GCCCAATTAT AATGATTAGT TATGCAGATG AAAATGAAGC AAAGGTGATT 1140

ACTTGGAAAA ACATAGATCT TCCATACGTT GAGGTTGTAT CAAGCGAGAG AGAGATGATA 1200

AAGAGATTTC TCAGGATTAT CAGGGAGAAG GATCCTGACA TTATAGTTAC TTATAATGGA 1260

GACTCATTCG ACTTCCCATA TTTAGCGAAA AGGGCAGAAA AACTTGGGAT TAAATTAACC 1320

ATTGGAAGAG ATGGAAGCGA GCCCAAGATG CAGAGAATAG GCGATATGAC GGCTGTAGAA 1380

GTCAAGGGAA GAATACATTT CGACTTGTAT CATGTAATAA CAAGGACAAT AAATCTCCCA 1440

ACATACACAC TAGAGGCTGT ATATGAAGCA ATTTTTGGAA AGCCAAAGGA GAAGGTATAC 1500

GCCGACGAGA TAGCAAAAGC CTGGGAAAGT GGAGAGAACC TTGAGAGAGT TGCCAAATAC 1560

TCGATGGAAG ATGCAAAGGC AACTTATGAA CTCGGGAAAG AATTCCTTCC AATGGAAATT 1620

CAGCTTTCAA GATTAGTTGG ACAACCTTTA TGGGATGTTT CAAGGTCAAG CACAGGGAAC 1680

CTTGTAGAGT GGTTCTTACT TAGGAAAGCC TACGAAAGAA ACGAAGTAGC TCCAAACAAG 1740

CCAAGTGAAG AGGAGTATCA AAGAAGGCTC AGGGAGAGCT ACACAGGTGG ATTCGTGCGC 1800

CTGGACGTGG CCTATCTCAG GGCCTTGTCC CTGGAGGTGG CCGAGGAGAT CGCCCGCCTC 1860

GAGGCCGAGG TCTTCCGCCT GGCCGGCCAC CCCTTCAACC TCAACTCCCG GGACCAGCTG 1920

GAAAGGGTCC TCTTTGACGA GCTAGGGCTT CCCGCCATCG GCAAGACGGA GAAGACCGGC 1980

AAGCGCTCCA CCAGCGCCGC CGTCCTGGAG GCCCTCCGCG AGGCCCACCC CATCGTGGAG 2040

AAGATCCTGC AGTACCGGGA GCTCACCAAG CTGAAGAGCA CCTACATTGA CCCCTTGCCG 2100

GACCTCATCC ACCCCAGGAC GGGCCGCCTC CACACCCGCT TCAACCAGAC GGCCACGGCC 2160

ACGGGCAGGC TAAGTAGCTC CGATCCCAAC CTCCAGAACA TCCCCGTCCG CACCCCGCTT 2220

GGGCAGAGGA TCCGCCGGGC CTTCATCGCC GAGGAGGGGT GGCTATTGGT GGCCCTGGAC 2280

TATAGCCAGA TAGAGCTCAG GGTGCTGGCC CACCTCTCCG GCGACGAGAA CCTGATCCGG 2340

GTCTTCCAGG AGGGGCGGGA CATCCACACG GAGACCGCCA GCTGGATGTT CGGCGTCCCC 2400

CGGGAGGCCG TGGACCCCCT GATGCGCCGG GCGGCCAAGA CCATCAACTT CGGGGTCCTC 2460

TACGGCATGT CGGCCCACCG CCTCTCCCAG GAGCTAGCCA TCCCTTACGA GGAGGCCCAG 2520

GCCTTCATTG AGCGCTACTT TCAGAGCTTC CCCAAGGTGC GGGCCTGGAT TGAGAAGACC 2580

CTGGAGGAGG GCAGGAGGCG GGGGTACGTG GAGACCCTCT TCGGCCGCCG CCGCTACGTG 2640

CCAGACCTAG AGGCCCGGGT GAAGAGCGTG CGGGAGGCGG CCGAGCGCAT GGCCTTCAAC 2700

ATGCCCGTCC AGGGCACCGC CGCCGACCTC ATGAAGCTGG CTATGGTGAA GCTCTTCCCC 2760

AGGCTGGAGG AAATGGGGGC CAGGATGCTC CTTCAGGTCC ACGACGAGCT GGTCCTCGAG 2820

GCCCCAAAAG AGAGGGCGGA GGCCGTGGCC CGGCTGGCCA AGGAGGTCAT GGAGGGGGTG 2880

TATCCCCTGG CCGTGCCCCT GGAGGTGGAG GTGGGGATAG GGGAGGACTG GCTCTCCGCC 2940

AAGGAGTGA 2949

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 910 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single strand
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Met Arg Gly Ser His His His His His His Ala Ala Asp Asp Asp Asp
1 5 10 15

Lys Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu
20 25 30

Leu Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys
35 40 45

Gly Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe
50 55 60

Ala Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile
65 70 75 80

Val Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly
85 90 95

Gly Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln
100 105 110

Leu Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu
115 120 125

Glu Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys
130 135 140

Lys Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys
145 150 155 160

Asp Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu
165 170 175

Gly Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg
180 185 190

Pro Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp
195 200 205

Asn Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu
210 215 220

Leu Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg
225 230 235 240

Leu Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu
245 250 255

Lys Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu
260 265 270

Val Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala
275 280 285

Phe Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu
290 295 300

Leu Glu Ser Pro Tyr Asp Asn Tyr Val Thr Ile Leu Asp Glu Glu Thr
305 310 315 320

Leu Lys Ala Trp Ile Ala Lys Leu Glu Lys Ala Pro Val Phe Ala Phe
325 330 335

Asp Thr Glu Thr Asp Ser Leu Asp Asn Ile Ser Ala Asn Leu Val Gly
340 345 350

Leu Ser Phe Ala Ile Glu Pro Gly Val Ala Ala Tyr Ile Pro Val Ala
355 360 365

His Asp Tyr Leu Asp Ala Pro Asp Gln Ile Ser Arg Glu Arg Ala Leu
370 375 380

Glu Leu Leu Lys Pro Leu Leu Glu Asp Glu Lys Ala Leu Lys Val Gly
385 390 395 400

Gln Asn Leu Lys Tyr Asp Arg Gly Ile Leu Ala Asn Tyr Gly Ile Glu
405 410 415

Leu Arg Gly Ile Ala Phe Asp Thr Met Leu Glu Ser Tyr Ile Leu Asn
420 425 430

Ser Val Ala Gly Arg His Asp Met Asp Ser Leu Ala Glu Arg Trp Leu
435 440 445

Lys His Lys Thr Ile Thr Phe Glu Glu Ile Ala Gly Lys Gly Lys Asn
450 455 460

Gln Leu Thr Phe Asn Gln Ile Ala Leu Glu Glu Ala Gly Arg Tyr Ala
465 470 475 480

Ala Glu Asp Ala Asp Val Thr Leu Gln Leu His Leu Lys Met Trp Pro
485 490 495

Asp Leu Gln Lys His Glu Arg Leu Leu Trp Leu Tyr Arg Glu Val Glu
500 505 510

Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly Val Arg
515 520 525

Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala Glu Glu
530 535 540

Val Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His Pro Phe
545 550 555 560

Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp Glu Leu
565 570 575

Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg Ser Thr
580 585 590

Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile Val Glu
595 600 605

Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr Tyr Ile
610 615 620

Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu His Thr
625 630 635 640

Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser Ser Asp
645 650 655

Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln Arg Ile
660 665 670

Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala Leu Asp
675 680 685

Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly Asp Glu
690 695 700

Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr Glu Thr
705 710 715 720

Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro Leu Met
725 730 735

Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly Met Ser
740 745 750

Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu Ala Gln
755 760 765

Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg Ala Trp
770 775 780

Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val Glu Thr
785 790 795 800

Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg Val Lys
805 810 815

```
Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro Val Gln
            820                 825                 830

Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu Phe Pro
        835                 840                 845

Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His Asp Glu
    850                 855                 860

Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala Arg Leu
865                 870                 875                 880

Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro Leu Glu
                885                 890                 895

Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
            900                 905                 910
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 910 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single strand
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Met Arg Gly Ser His His His His His His Ala Ala Asp Asp Asp Asp
1               5                   10                  15

Lys Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu
            20                  25                  30

Leu Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys
        35                  40                  45

Gly Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe
    50                  55                  60

Ala Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile
65                  70                  75                  80

Val Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly
                85                  90                  95

Gly Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln
            100                 105                 110

Leu Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu
        115                 120                 125

Glu Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys
    130                 135                 140
```

```
Lys Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys
145                 150                 155                 160

Asp Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu
                165                 170                 175

Gly Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg
            180                 185                 190

Pro Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp
        195                 200                 205

Asn Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu
    210                 215                 220

Leu Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg
225                 230                 235                 240

Leu Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu
                245                 250                 255

Lys Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu
            260                 265                 270

Val Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala
        275                 280                 285

Phe Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu
    290                 295                 300

Leu Glu Ser Pro Tyr Asp Asn Tyr Val Thr Ile Leu Asp Glu Glu Thr
305                 310                 315                 320

Leu Lys Ala Trp Ile Ala Lys Leu Glu Lys Ala Pro Val Phe Ala Phe
                325                 330                 335

Asp Thr Glu Thr Asp Ser Leu Asp Asn Ile Ser Ala Asn Leu Val Gly
            340                 345                 350

Leu Ser Phe Ala Ile Glu Pro Gly Val Ala Ala Tyr Ile Pro Val Ala
        355                 360                 365

His Asp Tyr Leu Asp Ala Pro Asp Gln Ile Ser Arg Glu Arg Ala Leu
    370                 375                 380

Glu Leu Leu Lys Pro Leu Leu Glu Asp Glu Lys Ala Leu Lys Val Gly
385                 390                 395                 400

Gln Asn Leu Lys Tyr Asp Arg Gly Ile Leu Ala Asn Tyr Gly Ile Glu
                405                 410                 415

Leu Arg Gly Ile Ala Phe Asp Thr Met Leu Glu Ser Tyr Ile Leu Asn
            420                 425                 430

Ser Val Ala Gly Arg His Asp Met Asp Ser Leu Ala Glu Arg Trp Leu
        435                 440                 445

Lys His Lys Thr Ile Thr Phe Glu Glu Ile Ala Gly Lys Gly Lys Asn
    450                 455                 460
```

Gln Leu Thr Phe Asn Gln Ile Ala Leu Glu Glu Ala Gly Arg Tyr Ala
465 470 475 480

Ala Glu Asp Ala Asp Val Thr Leu Gln Leu His Leu Lys Met Trp Pro
485 490 495

Asp Leu Gln Lys His Lys Gly Pro Leu Asn Val Phe Glu Asn Ile Glu
500 505 510

Met Pro Leu Val Pro Val Leu Ser Arg Ile Glu Arg Asn Gly Val Arg
515 520 525

Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala Glu Glu
530 535 540

Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His Pro Phe
545 550 555 560

Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp Glu Leu
565 570 575

Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg Ser Thr
580 585 590

Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile Val Glu
595 600 605

Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr Tyr Ile
610 615 620

Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu His Thr
625 630 635 640

Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser Ser Asp
645 650 655

Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln Arg Ile
660 665 670

Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala Leu Asp
675 680 685

Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly Asp Glu
690 695 700

Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr Glu Thr
705 710 715 720

Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro Leu Met
725 730 735

Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly Met Ser
740 745 750

Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu Ala Gln
755 760 765

Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg Ala Trp
770 775 780

```
Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val Glu Thr
785                 790                 795                 800

Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg Val Lys
                805                 810                 815

Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro Val Gln
            820                 825                 830

Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu Phe Pro
        835                 840                 845

Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His Asp Glu
    850                 855                 860

Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala Arg Leu
865                 870                 875                 880

Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro Leu Glu
                885                 890                 895

Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
            900                 905                 910
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 908 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
Met Arg Gly Ser His His His His His His Ala Ala Asp Asp Asp Asp
1               5                   10                  15

Lys Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu
            20                  25                  30

Leu Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys
        35                  40                  45

Gly Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe
    50                  55                  60

Ala Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile
65                  70                  75                  80

Val Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly
                85                  90                  95

Gly Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln
            100                 105                 110

Leu Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu
        115                 120                 125
```

Glu Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys
130 135 140

Lys Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys
145 150 155 160

Asp Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu
165 170 175

Gly Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg
180 185 190

Pro Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp
195 200 205

Asn Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu
210 215 220

Leu Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg
225 230 235 240

Leu Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu
245 250 255

Lys Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu
260 265 270

Val Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala
275 280 285

Phe Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu
290 295 300

Leu Glu Ser Pro Pro Val Gly Tyr Arg Ile Val Lys Asp Leu Val Glu
305 310 315 320

Phe Glu Lys Leu Ile Glu Lys Leu Arg Glu Ser Pro Ser Phe Ala Ile
325 330 335

Asp Leu Glu Thr Ser Ser Leu Asp Pro Phe Asp Cys Asp Ile Val Gly
340 345 350

Ile Ser Val Ser Phe Lys Pro Lys Glu Ala Tyr Tyr Ile Pro Leu His
355 360 365

His Arg Asn Ala Gln Asn Leu Asp Glu Lys Glu Val Leu Lys Lys Leu
370 375 380

Lys Glu Ile Leu Glu Asp Pro Gly Ala Lys Ile Val Gly Gln Asn Leu
385 390 395 400

Lys Phe Asp Tyr Lys Val Leu Met Val Lys Gly Val Glu Pro Val Pro
405 410 415

Pro His Phe Asp Thr Met Ile Ala Ala Tyr Leu Leu Glu Pro Asn Glu
420 425 430

Lys Lys Phe Asn Leu Asp Asp Leu Ala Leu Lys Phe Leu Gly Tyr Lys
435 440 445

```
Met Thr Ser Tyr Gln Glu Leu Met Ser Phe Ser Ser Pro Leu Phe Gly
    450                 455                 460

Phe Ser Phe Ala Asp Val Pro Val Glu Lys Ala Ala Asn Tyr Ser Cys
465                 470                 475                 480

Glu Asp Ala Asp Ile Thr Tyr Arg Leu Tyr Lys Ile Leu Ser Leu Lys
                485                 490                 495

Leu His Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu Val Glu Arg Pro
            500                 505                 510

Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly Val Arg Leu Asp
        515                 520                 525

Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala Glu Glu Ile Ala
    530                 535                 540

Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His Pro Phe Asn Leu
545                 550                 555                 560

Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp Glu Leu Gly Leu
                565                 570                 575

Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg Ser Thr Ser Ala
            580                 585                 590

Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile Val Glu Lys Ile
        595                 600                 605

Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr Tyr Ile Asp Pro
    610                 615                 620

Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu His Thr Arg Phe
625                 630                 635                 640

Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser Ser Asp Pro Asn
                645                 650                 655

Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln Arg Ile Arg Arg
            660                 665                 670

Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala Leu Asp Tyr Ser
        675                 680                 685

Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly Asp Glu Asn Leu
    690                 695                 700

Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr Glu Thr Ala Ser
705                 710                 715                 720

Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro Leu Met Arg Arg
                725                 730                 735

Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly Met Ser Ala His
            740                 745                 750

Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu Ala Gln Ala Phe
        755                 760                 765
```

Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg Ala Trp Ile Glu
770 775 780

Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val Glu Thr Leu Phe
785 790 795 800

Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg Val Lys Ser Val
805 810 815

Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro Val Gln Gly Thr
820 825 830

Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu Phe Pro Arg Leu
835 840 845

Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His Asp Glu Leu Val
850 855 860

Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala Arg Leu Ala Lys
865 870 875 880

Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro Leu Glu Val Glu
885 890 895

Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
900 905

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 908 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single strand
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Met Arg Gly Ser His His His His His His Ala Ala Asp Asp Asp Asp
1 5 10 15

Lys Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu
20 25 30

Leu Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys
35 40 45

Gly Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe
50 55 60

Ala Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile
65 70 75 80

Val Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly
85 90 95

Gly Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln
100 105 110

Leu Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu
115 120 125

Glu Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys
130 135 140

Lys Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys
145 150 155 160

Asp Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu
165 170 175

Gly Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg
180 185 190

Pro Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp
195 200 205

Asn Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu
210 215 220

Leu Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg
225 230 235 240

Leu Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu
245 250 255

Lys Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu
260 265 270

Val Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala
275 280 285

Phe Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu
290 295 300

Leu Glu Ser Pro Pro Val Gly Tyr Arg Ile Val Lys Asp Leu Val Glu
305 310 315 320

Phe Glu Lys Leu Ile Glu Lys Leu Arg Glu Ser Pro Ser Phe Ala Ile
325 330 335

Asp Leu Glu Thr Ser Ser Leu Asp Pro Phe Asp Cys Asp Ile Val Gly
340 345 350

Ile Ser Val Ser Phe Lys Pro Lys Glu Ala Tyr Tyr Ile Pro Leu His
355 360 365

His Arg Asn Ala Gln Asn Leu Asp Glu Lys Glu Val Leu Lys Lys Leu
370 375 380

Lys Glu Ile Leu Glu Asp Pro Gly Ala Lys Ile Val Gly Gln Asn Leu
385 390 395 400

Lys Phe Asp Tyr Lys Val Leu Met Val Lys Gly Val Glu Pro Val Pro
405 410 415

Pro His Phe Asp Thr Met Ile Ala Ala Tyr Leu Leu Glu Pro Asn Glu
420 425 430

Lys Lys Phe Asn Leu Asp Asp Leu Ala Leu Lys Phe Leu Gly Tyr Lys
435 440 445

Met Thr Ser Tyr Gln Glu Leu Met Ser Phe Ser Ser Pro Leu Phe Gly
450 455 460

Phe Ser Phe Ala Asp Val Pro Val Glu Lys Ala Ala Asn Tyr Ser Cys
465 470 475 480

Glu Asp Ala Asp Ile Thr Tyr Arg Leu Tyr Lys Ile Leu Ser Leu Lys
485 490 495

Leu His Glu Ala Asp Leu Glu Asn Val Phe Tyr Lys Ile Glu Met Pro
500 505 510

Leu Val Ser Val Leu Ala Arg Met Glu Leu Asn Gly Val Arg Leu Asp
515 520 525

Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala Glu Glu Ile Ala
530 535 540

Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His Pro Phe Asn Leu
545 550 555 560

Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp Glu Leu Gly Leu
565 570 575

Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg Ser Thr Ser Ala
580 585 590

Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile Val Glu Lys Ile
595 600 605

Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr Tyr Ile Asp Pro
610 615 620

Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu His Thr Arg Phe
625 630 635 640

Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser Ser Asp Pro Asn
645 650 655

Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln Arg Ile Arg Arg
660 665 670

Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala Leu Asp Tyr Ser
675 680 685

Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly Asp Glu Asn Leu
690 695 700

Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr Glu Thr Ala Ser
705 710 715 720

Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro Leu Met Arg Arg
725 730 735

Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly Met Ser Ala His
740 745 750

Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu Ala Gln Ala Phe
755 760 765

Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg Ala Trp Ile Glu
770 775 780

Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val Glu Thr Leu Phe
785 790 795 800

Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg Val Lys Ser Val
805 810 815

Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro Val Gln Gly Thr
820 825 830

Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu Phe Pro Arg Leu
835 840 845

Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His Asp Glu Leu Val
850 855 860

Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala Arg Leu Ala Lys
865 870 875 880

Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro Leu Glu Val Glu
885 890 895

Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
900 905

(2) INFORMATION FORSEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 949 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single strand
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Arg Gly Ser His His His His His His Ala Ala Asp Asp Asp Asp
1 5 10 15

Lys Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu
20 25 30

Leu Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys
35 40 45

Gly Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe
50 55 60

Ala Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile
65 70 75 80

Val Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly
85 90 95

Gly Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln
100 105 110

Leu Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu
115 120 125

Glu Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys
130 135 140

Lys Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys
145 150 155 160

Asp Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu
165 170 175

Gly Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg
180 185 190

Pro Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp
195 200 205

Asn Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu
210 215 220

Leu Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg
225 230 235 240

Leu Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu
245 250 255

Lys Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu
260 265 270

Val Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala
275 280 285

Phe Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu
290 295 300

Leu Glu Ser Pro His Pro Ala Val Val Asp Ile Phe Glu Tyr Asp Ile
305 310 315 320

Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile Pro Met Glu
325 330 335

Gly Glu Glu Glu Leu Lys Ile Leu Ala Phe Asp Ile Glu Thr Leu Tyr
340 345 350

His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met Ile Ser Tyr
355 360 365

Ala Asp Glu Asn Glu Ala Lys Val Ile Thr Trp Lys Asn Ile Asp Leu
370 375 380

Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile Lys Arg Phe
385 390 395 400

Leu Arg Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Val Thr Tyr Asn
405 410 415

Gly Asp Ser Phe Asp Phe Pro Tyr Leu Ala Lys Arg Ala Glu Lys Leu
420 425 430

Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro Lys Met Gln
435 440 445

Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg Ile His Phe
450 455 460

Asp Leu Tyr His Val Ile Thr Arg Thr Ile Asn Leu Pro Thr Tyr Thr
465 470 475 480

Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys Glu Lys Val
485 490 495

Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Ser Gly Glu Asn Leu Glu
500 505 510

Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr Tyr Glu Leu
515 520 525

Gly Lys Glu Phe Leu Pro Met Glu Ile Gln Leu Ser Glu Arg Leu Leu
530 535 540

Trp Leu Tyr Arg Glu Val Glu Arg Pro Leu Ser Ala Val Leu Ala His
545 550 555 560

Met Glu Ala Thr Gly Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu
565 570 575

Ser Leu Glu Val Ala Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe
580 585 590

Arg Leu Ala Gly His Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu
595 600 605

Arg Val Leu Phe Asp Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu
610 615 620

Lys Thr Gly Lys Arg Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg
625 630 635 640

Glu Ala His Pro Ile Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr
645 650 655

Lys Leu Lys Ser Thr Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro
660 665 670

Arg Thr Gly Arg Leu His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr
675 680 685

Gly Arg Leu Ser Ser Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg
690 695 700

Thr Pro Leu Gly Gln Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly
705 710 715 720

Trp Leu Leu Val Ala Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu
725 730 735

Ala His Leu Ser Gly Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly
740 745 750

Arg Asp Ile His Thr Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg
755 760 765

Glu Ala Val Asp Pro Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe
770 775 780

Gly Val Leu Tyr Gly Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala
785 790 795 800

Ile Pro Tyr Glu Glu Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser
805 810 815

Phe Pro Lys Val Arg Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg
820 825 830

Arg Arg Gly Tyr Val Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro
835 840 845

Asp Leu Glu Ala Arg Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met
850 855 860

Ala Phe Asn Met Pro Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu
865 870 875 880

Ala Met Val Lys Leu Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met
885 890 895

Leu Leu Gln Val His Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg
900 905 910

Ala Glu Ala Val Ala Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr
915 920 925

Pro Leu Ala Val Pro Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp
930 935 940

Leu Ser Ala Lys Glu
945

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 982 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single strand
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Met Arg Gly Ser His His His His His His Ala Ala Asp Asp Asp Asp
1 5 10 15

Lys Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu
20 25 30

Leu Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys
35 40 45

Gly Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe
50 55 60

Ala Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile
65 70 75 80

Val Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly
85 90 95

Gly Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln
100 105 110

Leu Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu
115 120 125

Glu Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys
130 135 140

Lys Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys
145 150 155 160

Asp Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu
165 170 175

Gly Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg
180 185 190

Pro Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp
195 200 205

Asn Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu
210 215 220

Leu Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg
225 230 235 240

Leu Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu
245 250 255

Lys Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu
260 265 270

Val Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala
275 280 285

Phe Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu
290 295 300

Leu Glu Ser Pro Val Arg Glu His Pro Ala Val Val Asp Ile Phe Glu
305 310 315 320

Tyr Asp Ile Pro Phe Ala Lys Arg Tyr Leu Ile Asp Lys Gly Leu Ile
325 330 335

Pro Met Glu Gly Glu Glu Glu Leu Lys Ile Leu Ala Phe Asp Ile Glu
340 345 350

Thr Leu Tyr His Glu Gly Glu Glu Phe Gly Lys Gly Pro Ile Ile Met
355 360 365

Ile Ser Tyr Ala Asp Glu Asn Glu Ala Lys Val Ile Thr Trp Lys Asn
370 375 380

Ile Asp Leu Pro Tyr Val Glu Val Val Ser Ser Glu Arg Glu Met Ile
385 390 395 400

Lys Arg Phe Leu Arg Ile Ile Arg Glu Lys Asp Pro Asp Ile Ile Val
405 410 415

Thr Tyr Asn Gly Asp Ser Phe Asp Phe Pro Tyr Leu Ala Lys Arg Ala
420 425 430

Glu Lys Leu Gly Ile Lys Leu Thr Ile Gly Arg Asp Gly Ser Glu Pro
435 440 445

Lys Met Gln Arg Ile Gly Asp Met Thr Ala Val Glu Val Lys Gly Arg
450 455 460

Ile His Phe Asp Leu Tyr His Val Ile Thr Arg Thr Ile Asn Leu Pro
465 470 475 480

Thr Tyr Thr Leu Glu Ala Val Tyr Glu Ala Ile Phe Gly Lys Pro Lys
485 490 495

Glu Lys Val Tyr Ala Asp Glu Ile Ala Lys Ala Trp Glu Ser Gly Glu
500 505 510

Asn Leu Glu Arg Val Ala Lys Tyr Ser Met Glu Asp Ala Lys Ala Thr
515 520 525

Tyr Glu Leu Gly Lys Glu Phe Leu Pro Met Glu Ile Gln Leu Ser Arg
530 535 540

Leu Val Gly Gln Pro Leu Trp Asp Val Ser Arg Ser Ser Thr Gly Asn
545 550 555 560

Leu Val Glu Trp Phe Leu Leu Arg Lys Ala Tyr Glu Arg Asn Glu Val
565 570 575

Ala Pro Asn Lys Pro Ser Glu Glu Glu Tyr Gln Arg Arg Leu Arg Glu
580 585 590

Ser Tyr Thr Gly Gly Phe Val Arg Leu Asp Val Ala Tyr Leu Arg Ala
595 600 605

Leu Ser Leu Glu Val Ala Glu Glu Ile Ala Arg Leu Glu Ala Glu Val
610 615 620

Phe Arg Leu Ala Gly His Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu
625 630 635 640

Glu Arg Val Leu Phe Asp Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr
645 650 655

Glu Lys Thr Gly Lys Arg Ser Thr Ser Ala Ala Val Leu Glu Ala Leu
660 665 670

Arg Glu Ala His Pro Ile Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu
675 680 685

Thr Lys Leu Lys Ser Thr Tyr Ile Asp Pro Leu Pro Asp Leu Ile His
690 695 700

Pro Arg Thr Gly Arg Leu His Thr Arg Phe Asn Gln Thr Ala Thr Ala
705 710 715 720

Thr Gly Arg Leu Ser Ser Ser Asp Pro Asn Leu Gln Asn Ile Pro Val
725 730 735

Arg Thr Pro Leu Gly Gln Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu
740 745 750

Gly Trp Leu Leu Val Ala Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val
755 760 765

Leu Ala His Leu Ser Gly Asp Glu Asn Leu Ile Arg Val Phe Gln Glu
770 775 780

Gly Arg Asp Ile His Thr Glu Thr Ala Ser Trp Met Phe Gly Val Pro
785 790 795 800

Arg Glu Ala Val Asp Pro Leu Met Arg Arg Ala Ala Lys Thr Ile Asn
805 810 815

Phe Gly Val Leu Tyr Gly Met Ser Ala His Arg Leu Ser Gln Glu Leu
820 825 830

Ala Ile Pro Tyr Glu Glu Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln
835 840 845

Ser Phe Pro Lys Val Arg Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly
850 855 860

Arg Arg Arg Gly Tyr Val Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val
865 870 875 880

Pro Asp Leu Glu Ala Arg Val Lys Ser Val Arg Glu Ala Ala Glu Arg
885 890 895

Met Ala Phe Asn Met Pro Val Gln Gly Thr Ala Ala Asp Leu Met Lys
900 905 910

Leu Ala Met Val Lys Leu Phe Pro Arg Leu Glu Glu Met Gly Ala Arg
915 920 925

Met Leu Leu Gln Val His Asp Glu Leu Val Leu Glu Ala Pro Lys Glu
930 935 940

Arg Ala Glu Ala Val Ala Arg Leu Ala Lys Glu Val Met Glu Gly Val
945 950 955 960

Tyr Pro Leu Ala Val Pro Leu Glu Val Glu Val Gly Ile Gly Glu Asp
965 970 975

Trp Leu Ser Ala Lys Glu
980

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single strand
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(ix) CHARACTERISTIC:
(A) NAME/KEY: CDS
(B) POSITION:1..66

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GAA TTC ATG AGG GGC TCG CAT CAC CAT CAC CAT CAC GCT GCT GAC GAT 48

GAC GAT AAA ATG AGG GGC 66

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Met Arg Gly Ser His His His His His His Ala Ala Asp Asp Asp Asp
1 5 10 15

Lys Met Arg Gly
20